Portner
520946
Att#6

```
=> fil reg; e cleavase bn/cn 5
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  conducting SmartSELECT searches.                    -key terms


E1            1      CLEAVAMINE-18-CARBOXYLIC ACID, METHYL ESTER, MONOACETA
                     TE/CN
E2            1      CLEAVASE BB/CN
E3            1 --> CLEAVASE BN/CN
E4            1      CLEAVASE BX/CN
E5            1      CLEAVELANDITE/CN

=> e thermus aquaticus dna polymerase/cn 5
E1            1      THERMPHOS SPR/CN
E2            1      THERMTEX/CN
E3            0 --> THERMUS AQUATICUS DNA POLYMERASE/CN
E4            1      THERMUS AQUATICUS EXTRACELLULAR SERINE PROTEINASE/CN
E5            1      THERMUS THERMOPHILUS EXTRACELLULAR ENDONUCLEASE/CN

=> e dna polymerase, thermus aquaticus/cn 5
E1            1      DNA POLYMERASE III SUBUNIT .PSI. (ESCHERICHIA COLI GEN
                     E HOLD)/CN
E2            1      DNA POLYMERASE III SUBUNIT .THETA. (ESCHERICHIA COLI G
                     ENE HOLE)/CN
E3            0 --> DNA POLYMERASE, THERMUS AQUATICUS/CN
E4            1      DNA POLYMERASE-.ALPHA. CATALYTIC SUBUNIT P180 (MOUSE)/
                     CN
E5            1      DNA POLYMERASE-.ALPHA.-PRIMASE SUBUNIT P68 (CALF THYMU
                     S N-TERMINAL FRAGMENT)/CN

=> e escherichia coli exo iii/cn 5
E1            1      ESCHERICHIA COLI ENDONUCLEASE II/CN
E2            1      ESCHERICHIA COLI ENDONUCLEASE III/CN
E3            0 --> ESCHERICHIA COLI EXO III/CN
E4            1      ESCHERICHIA COLI EXO-RNASE II/CN
E5            1      ESCHERICHIA COLI EXONUCLEASE I/CN

=> e escherichia coli exonuclease iii/cn 5
E1            1      ESCHERICHIA COLI EXONUCLEASE I/CN
E2            1      ESCHERICHIA COLI EXONUCLEASE II/CN
E3            1 --> ESCHERICHIA COLI EXONUCLEASE III/CN
E4            1      ESCHERICHIA COLI EXONUCLEASE IV/CN
E5            1      ESCHERICHIA COLI EXONUCLEASE V/CN
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=> e saccharomyces cerevisiae rad1/cn 5
E1            1      SACCHAROMYCES CEREVISIAE METALLOPROTEINASE/CN
E2            1      SACCHAROMYCES CEREVISIAE PROPROTEINASE A/CN
E3            0 --> SACCHAROMYCES CEREVISIAE RAD1/CN
E4            1      SACCHAROMYCES CEREVISIAE SITE-SPECIFIC ENDODEOXYRIBONU
                     CLEASE SCE I/CN
E5            1      SACCHAROMYCES CEREVISIAE TRYPTOPHAN TRANSFER RNA I/CN

=> s (cleavase bn or escherichia coli exonuclease iii)/cn; fil ca,caplus
              1 CLEAVASE BN/CN
              1 ESCHERICHIA COLI EXONUCLEASE III/CN
L1            2 (CLEAVASE BN OR ESCHERICHIA COLI EXONUCLEASE III)/CN


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=> s cleav? or enzyme# or nuclease# or (aquaticus or thermophil?)(w)(dna
or deoxyribonucleic)(1w)polymerase# or coli(w)(exoiii or exo3 or exo?(w)(3
or iii) or cerevis?(w)((rad1 or rad 1)(a)(rad10 or rad 10))
UNMATCHED LEFT PARENTHESIS 'W)(EXOIII'
The number of right parentheses in a query must be equal to the
number of left parentheses.

=> s cleav? or enzyme# or nuclease# or (aquaticus or thermophil?)(w)(dna
or deoxyribonucleic)(1w)polymerase# or coli(w)(exoiii or exo3 or exo?(w)(3
or iii)) or cerevis?(w)((rad1 or rad 1)(a)(rad10 or rad 10))
L2       655957 FILE CA
L3       659967 FILE CAPLUS

TOTAL FOR ALL FILES
L4      1315924 CLEAV? OR ENZYME# OR NUCLEASE# OR (AQUATICUS OR THERMOPHIL
                ?)(W)(DNA OR DEOXYRIBONUCLEIC)(1W) POLYMERASE# OR COLI(W)(
                EXOIII OR EXO3 OR EXO?(W)(3 OR III)) OR CEREVIS?(W)((RAD1
                OR RAD 1)(A)(RAD10 OR RAD 10))

=> fil reg
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=> fil reg; d que l1; fil ca,caplus
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  conducting SmartSELECT searches.


L1             2 SEA FILE=REGISTRY (CLEAVASE BN OR ESCHERICHIA COLI EXONUC
                 LEASE III)/CN


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=> s l1 or cleavase(1w)bn or (aquaticus or thermophil?)(w)(dna or
deoxyribonucleic)(1w)polymerase# or coli(w)(exoiii or exo3 or exo?(w)(3 or
iii)) or cerevis?(w)((rad1 or rad 1)(a)(rad10 or rad 10))
L2           693 FILE CA
L3           696 FILE CAPLUS

TOTAL FOR ALL FILES
L4          1389 L1 OR CLEAVASE(1W) BN OR (AQUATICUS OR THERMOPHIL?)(W)(DNA
                  OR DEOXYRIBONUCLEIC)(1W) POLYMERASE# OR COLI(W)(EXOIII OR
                  EXO3 OR EXO?(W)(3 OR III)) OR CEREVIS?(W)((RAD1 OR RAD 1)
                 (A)(RAD10 OR RAD 10))

=> fil reg; e dutp/cn 5
FILE 'REGISTRY' ENTERED AT 16:31:55 ON 08 MAY 96
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  Please note that search-term pricing does apply when
  conducting SmartSELECT searches.


E1             1      DUTOM/CN
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E2            1      DUTOMYCIN/CN
E3            1 --> DUTP/CN
E4            1      DUTP-21-SS-BIOTIN/CN
E5            1      DUTPASE (EQUINE HERPES VIRUS TYPE 4 CLONE PUC4S GENE B
                     3)/CN

=> s e3
L5            1 DUTP/CN

=> e "7-deaza-datp"/cn 5
E1            1      7-DEAZA-8-AZAGUANOSINE/CN
E2            1      7-DEAZA-AMP/CN
E3            0 --> 7-DEAZA-DATP/CN
E4            1      7-DEAZAADENINE/CN
E5            1      7-DEAZAADENOSINE/CN

=> e "7-deaza-deoxyadenosine triphosphate"/cn 5
E1            1      7-DEAZA-8-AZAGUANOSINE/CN
E2            1      7-DEAZA-AMP/CN
E3            0 --> 7-DEAZA-DEOXYADENOSINE TRIPHOSPHATE/CN
E4            1      7-DEAZAADENINE/CN
E5            1      7-DEAZAADENOSINE/CN

=> e "7-deazadeoxyadenosine triphosphate"/cn 5
E1            1      7-DEAZAADENOSINE-7-CARBOXAMIDE/CN
E2            1      7-DEAZADEOXYADENOSINE/CN
E3            0 --> 7-DEAZADEOXYADENOSINE TRIPHOSPHATE/CN
E4            1      7-DEAZAGUANINE/CN
E5            1      7-DEAZAGUANOSINE/CN

=> e "7-deazadeoxyguanosine triphosphate"/cn 5
E1            1      7-DEAZAADENOSINE-7-CARBOXAMIDE/CN
E2            1      7-DEAZADEOXYADENOSINE/CN
E3            0 --> 7-DEAZADEOXYGUANOSINE TRIPHOSPHATE/CN
E4            1      7-DEAZAGUANINE/CN
E5            1      7-DEAZAGUANOSINE/CN

=> e "7-deaza-dgtp"/cn 5
E1            1      7-DEAZA-8-AZAGUANOSINE/CN
E2            1      7-DEAZA-AMP/CN
E3            0 --> 7-DEAZA-DGTP/CN
E4            1      7-DEAZAADENINE/CN
E5            1      7-DEAZAADENOSINE/CN

=> fil ca,caplus
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```

=> s l4 and (l5 or 7(w)deaza? or dutp or d utp or deoxyuridinetriphosphate or deoxyuridine? or deoxy uridine?)
L6 15 FILE CA
L7 15 FILE CAPLUS

TOTAL FOR ALL FILES
L8 30 L4 AND (L5 OR 7(W) DEAZA? OR DUTP OR D UTP OR DEOXYURIDINE TRIPHOSPHATE OR DEOXYURIDINE? OR DEOXY URIDINE?)

=> dup rem l8; d 1-15 .bevstr; fil biosi,medl,embas,lifesci,biotechds,wpids,confsci,dissabs,scisearch
PROCESSING COMPLETED FOR L8
L9 15 DUP REM L8 (15 DUPLICATES REMOVED)

L9 ANSWER 1 OF 15 CA COPYRIGHT 1996 ACS DUPLICATE 1
AN 124:195956 CA
TI PCR with fluorescently labeled nucleotide triphosphates for DNA sequence determination
IN Ikeda, Katsunori; Inoe, Hiroaki; Oka, Masanori; Kawamura, Yoshihisa
PA Toyo Boseki, Japan
SO Jpn. Kokai Tokkyo Koho, 8 pp.
CODEN: JKXXAF
PI JP 07313198 A2 951205 Heisei
AI JP 94-108504 940523
DT Patent
LA Japanese
OS MARPAT 124:195956
AB Disclosed is a method using unlabeled nucleotide triphosphates, DNA synthetase, and biotin-, digoxigenin-, enzyme- or fluorescent dye-labeled nucleotide triphosphates for DNA sequence detn. The nucleotide triphosphate deriv. is e.g. dideoxy-7-**deaza** nucleotide-5'-triphosphate, and the labeled nucleotide triphosphate is e.g. 7-[N-biotinyl-(3-amino-1-propyl)]-2',3'-dideoxy-**7-deazaguanosine**-5'-triphosphate, 7-[N-biotinyl-(3-amino-1-propyl)]-2',3'-dideoxy-7-**deazaadenosine**-5'-triphosphate, 5-[N-biotinyl-(3-amino-1-propyl)]-2',3'-dideoxyuridine-5'-triphosphate, and 5-[N-biotinyl-(3-amino-1-propyl)]-2',3'-dideoxycytidine-5'-triphosphate. Alk. phosphatase, avidin, 1,2-dihydroxycetane, 5-bromo-4-chloro-3-indolyl phosphate, or NitroBlue tetrazolium is used for signal generation. 5'.fwdarw.3' Exonuclease, 3'.fwdarw.5' exonuclease, Thermus thermophilus-derived Tth DNA polymerase, Thermus aquaticus-derived Taq DNA polymerase, and Pyrococcus furiosus-derived DNA polymerase are usable for the disclosed method.
IT **79393-91-2**, 3'.fwdarw.5' Exonuclease
RL: ARU (Analytical role, unclassified); ANST (Analytical study)
(PCR with unlabeled nucleotide triphosphates, DNA synthetase, and biotin-, digoxigenin-, enzyme- or fluorescent dye-labeled nucleotide triphosphates for DNA sequence detn)

L9 ANSWER 2 OF 15 CA COPYRIGHT 1996 ACS DUPLICATE 2

AN 122:257225 CA
TI In situ transcription with Tth DNA polymerase and fluorescent nucleotides
AU Chang, Henry
CS Naval Medical Research Institute, Code 63, Bethesda, MD, 20889-5607, USA
SO J. Immunol. Methods (1994), 176(2), 235-43
CODEN: JIMMBG; ISSN: 0022-1759
DT Journal
LA English
AB We and others have described methods to label specific nucleic acid sequences in fixed cells by reverse in situ transcription (IST). These methods are simple alternatives to the tedious steps of in situ hybridization with labeled probes. We have favored use of thermostable DNA polymerases after heat denaturation of the template secondary structure, accompanied by synthesis of cDNA from an annealed primer, but the approach has been limited by the low reverse transcriptase (RT) activity of Taq polymerase and by delayed detection methods. We have improved the technique by the use of recombinant Thermus thermophilus (rTth) DNA polymerase and fluorescein-12-**dUTP** (FIST). Jurkat T lymphocytes were stimulated with ionomycin and phorbol myristate acetate to produce interleukin-2 (IL-2) mRNA in vitro overnight. They were cytospun onto slides and fixed in 70% ethanol + 30% DEPC-treated water, acetone, and were air-dried. The slides were placed on a temp.-controlled heating block, and the cell spot was covered with a plastic coverslip. The temp. was raised to 95.degree.C, and 5-10 .mu.l of modified Perkin-Elmer/Cetus rTth RT reaction mix was injected under the edge of the coverslip. Each 10 .mu.l of mix in DEPC-water contained 10 mM Tris-HCl, pH 8.3, 90 mM KCl, 1 mM MnCl2, 1 mM dithiothreitol, 10 U placental RNase inhibitor, 0.125 mM dA,C,GTPs, 0.1 mM fluorescein-12-**dUTP**, 2 U rTth DNA polymerase, and 4 pM 22-mer oligonucleotide primer, which spanned the second intron of IL-2. After 3 min at 95.degree.C, 1 min at 50.degree.C, and 10 min at 72.degree.C, the slides were washed in 0.5.times. phosphate-buffered saline, pH 7.0 at 42.degree.C, in 70% ethanol, 100% ethanol, and were air-dried. The cells were mounted in antifade soln. (2% n-Pr gallate in 70% glycerol), and could be viewed immediately by fluorescence microscopy. Image anal. showed that stimulated Jurkat cells were brighter than uninduced controls or those treated with RNase or those treated without polymerase or primer. FIST appears to be useful for the detection of specific mRNAs in single cells.

L9 ANSWER 3 OF 15 CA COPYRIGHT 1996 ACS DUPLICATE 3
AN 119:199010 CA
TI Detection of 5-bromo-2-**deoxyuridine** (BrdUrd) incorporation with monoclonal anti-BrdUrd antibody after deoxyribonuclease treatment
AU Takagi, Shuji; McFadden, Marcia L.; Humphreys, Robert E.; Woda, Bruce A.; Sairenji, Takeshi
CS Med. Sch., Univ. Massachusetts, Worcester, MA, 01655, USA
SO Cytometry (1993), 14(6), 640-8

CODEN: CYTODQ; ISSN: 0196-4763
DT Journal
LA English
AB The effects of DNases on the detection of 5-bromo-2-**deoxyuridine** (BrdUrd) by anti-BrdUrd monoclonal antibodies (mAbs) were studied. After DNase I treatment, BrdUrd was detected in cells fixed on slides with the anti-BrdUrd mAbs, B44 and BMC9318. The level of detection related to the degree of DNA digestion. DNA digestion of 25-75% resulted in levels of staining comparable to control prepns. in which DNA was denatured by heating with formamide. Staining with the mAbs of DNase I-treated cells was abolished with S1 nuclease, a single-stranded DNA-specific nuclease. When exonuclease III was used after DNase I treatment, the staining intensity of cells fixed on slides increased, and BrdUrd could be detected in suspended cells by flow cytometry. Since this enzymic method leading to the detection of BrdUrd does not involve cell loss, or destruction of either cellular morphol. or epitope reactivity, as occurs with traditional DNA denaturation procedures, it is useful for kinetic studies of phenotypically mixed populations. Furthermore, staining with anti-BrdUrd mAb of cells treated with exonuclease III offers a simple approach to quantitation of apoptotic cells, in which an endogenous endonuclease is activated.
IT **9037-44-9**, Exonuclease III
RL: ANST (Analytical study)
(bromodeoxyuridine detection with monoclonal antibodies after DNase treatment in relation to)

L9 ANSWER 4 OF 15 CA COPYRIGHT 1996 ACS DUPLICATE 4
AN 119:132483 CA
TI Selective digestion of mouse chromosomes with restriction endonucleases. Oligonucleotide priming of single-stranded DNA produced with exonuclease III
AU Gosalvez, J.; Lopez-Fernandez, C.; Garcia de la Vega, C.; Mezzanotte, R.; Fernandez, J. L.; Goyanes, V.
CS Fac. Cienc., Univ. Auton. Madrid, Madrid, 28049, Spain
SO Genome (1993), 36(2), 230-4
CODEN: GENOE3; ISSN: 0831-2796
DT Journal
LA English
AB L-929 mouse chromosomes prepd. for electron microscopy have been treated with MspI, EcoRI, and HaeIII restriction endonucleases (REs). RE-induced nicks were amplified with exonuclease III to obtain single-stranded DNA (ssp-DNA) motifs. The ss-DNA produced was enough to permit hybridization of a series of random oligonucleotides. These can be used as primers, which are extended by the Klenow fragment using non-isotopic labeled **dUTP**. The incorporation of biotinylated **dUTP** is detected with a gold-tagged streptavidin as the reporter mol. This allows, in mouse chromosomes, the detection of different rates of sensitivity to the digestion with specific REs in distinct intraheterochromatic DNA subsets. In addn., these results show that enzymic prodn. of ss-DNA seems to be adequate for electron microscopy work since the

chromatin fiber is preserved better than in denatured DNA produced with heat, NaOH, or formamide.

IT **9037-44-9**, Exonuclease III
RL: USES (Uses)
(chromosomes of L-929 mouse cells selective digestion with restriction endonucleases and, oligonucleotide priming of single-stranded DNA after, intraheterochromatic DNA subsets in relation to)

L9 ANSWER 5 OF 15 CA COPYRIGHT 1996 ACS DUPLICATE 5
AN 119:1900 CA
TI Low incorporation of dUMP by some thermostable DNA polymerases may limit their use in PCR amplifications
AU Slupphaug, Geir; Alseth, Ingrun; Eftedal, Ingrid; Volden, Gunnar; Krokan, Hans E.
CS UNIGEN Cent. Mol. Biol., Univ. Trondheim, N-7005, Norway
SO Anal. Biochem. (1993), 211(1), 164-93
CODEN: ANBCA2; ISSN: 0003-2697
DT Journal
LA English
AB Incorporation of dUMP instead of dTMP is frequently used to control carryover contamination during PCR amplifications. Four thermostable DNA polymerases were tested for their ability to utilize **dUTP** as a substrate in PCR. Amplification of products in the presence of **dUTP** instead of dTTP was good with Thermus **aquaticus DNA polymerase** but highly inefficient with three other thermostable DNA polymerases. The latter was due to: (a) lower incorporation of dUMP relative to dTMP, (b) increased proofreading toward dUMP in DNA, (c) relative termination at dUMP residues as verified by sequencing reactions in the presence of **dUTP**, and (d) thermostable dUTPase activity in the com. enzyme prepn. The last point only applies to Pyrococcus furiosus DNA polymerase. This study demonstrates that various thermostable DNA polymerases utilize dTTP and **dUTP** with highly different efficiencies and thus the choice of DNA polymerase may be crit. for amplification of DNA.

IT **1173-82-6, DUTP**
RL: PROC (Process)
(differential utilization of, by thermostable DNA polymerase from Thermus aquaticus and Thermococcus litoralis and Pyrococcus furiosus, in PCR)

L9 ANSWER 6 OF 15 CA COPYRIGHT 1996 ACS DUPLICATE 6
AN 119:220580 CA
TI Roles of uracil-DNA glycosylase and apyrimidinic endonucleases in the molecular 5-bromo-2'-**deoxyuridine** photosensitization in Escherichia coli K-12
AU Yamamoto, Yoko; Fujiwara, Yoshisada
CS Sch. Med., Kobe Univ., Kobe, 650, Japan
SO Photochem. Photobiol. (1993), 58(1), 66-70
CODEN: PHCBAP; ISSN: 0031-8655
DT Journal
LA English

AB The mol. mechanism for 5-bromo-2'-**deoxyuridine** (BrdU) photosensitization was studied in thymine-requiring wild-type and uracil-DNA glycosylase (UDG)-deficient ung mutant cells of Escherichia coli K-12. Wild-type cells were more sensitive to BrdU photosensitization than ung mutant cells. UV-induced the identical nos. of alk. sucrose single-strand breaks (SSB) in 5-bromouracil-DNA (BrU-DNA) of both the wild-type and ung mutant. The ung mutant cells repaired SSB almost completely, whereas the wild-type cells with UDG produced more adverse SSB by 90 min after UV. Neutral agarose gel electrophoresis of minipreps indicated that UV induced (1) more smears of host BrU-DNA possibly by more double-strand breaks (DSB) and (2) a greater decline of pBR322 Form I BrU-DNA in the wild-type cells than the ung cells. These results indicated a greater induction of SSB by apyrimidinic (AP) endonucleases in wild-type cells. The ung/wild ratios (=1.7-1.9) for cellular and plasmid BrdU sensitizations after growth in 50% BrdU were similar. The extents of UDG-dependent and UDG-independent sensitizations in wild-type cells were .apprx.40 and .apprx.60%, resp. The xth nfo double mutant defective in both exonuclease III and endonuclease IV was more sensitive to BrdU photosensitization than the wild type, indicating that an excess of AP sites remaining after uracil excision in the xth nfo mutant causes a greater BrdU photosensitization than SSB by AP endonucleases in wild-type cells. Conversely, the xth nfo ung triple mutant was more resistant to BrdU photosensitization than the xth nfo double mutant, so that UV-induced uracil residues in the BrU-DNA are tolerated and do not appear to be directly responsible for BrdU photosensitization.

IT **9037-44-9**, Exonuclease III
RL: BIOL (Biological study)
(in bromodeoxyuridine photosensitization of Escherichia coli)

L9 ANSWER 7 OF 15 CA COPYRIGHT 1996 ACS DUPLICATE 7
AN 117:21509 CA
TI Sensitive detection process for nucleic acids
IN Kessler, Christoph; Rueger, Ruediger; Seibl, Rudolf; Kruse-Mueller, Cornelia; Berner, Sibylle
PA Boehringer Mannheim G.m.b.H., Germany
SO PCT Int. Appl., 53 pp.
CODEN: PIXXD2
PI WO 9206216 A1 920416
DS W: AU, BG, BR, CA, CS, FI, HU, JP, KR, NO, PL, RO, SU, US
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, NL, SE
AI WO 91-EP1898 911004
PRAI DE 90-4032024 901009
DE 90-4038804 901205
DE 90-4041608 901222
DT Patent
LA German
AB A nucleic acid A is specifically detected in a sample by (1) reacting the sample with .gtoreq.1 labeled mononucleoside triphosphates, a primer, and .gtoreq.1 enzyme (polymerase) which catalyzes the formation of a labeled nucleic acid B, complementary to A, contg. this nucleotide, and nonthermally denaturing B; (2)

reacting the sample with a nucleic acid probe C, contg. an immobilizable group, which is adequately complementary to B; (3) contacting hybrid nucleic acid D, formed from hybridization of B and C, with a solid phase capable of binding C, sepg. the solid and liq. phases, and detecting the label bound to the solid phase. Thus, target hepatitis B virus DNA was amplified by PCR using Thermus **aquaticus DNA polymerase** and primers which bind to positions 1937-1960 and 2434-2460 in the presence of digoxigenin 11-(2'-**deoxyuridine** 5'-triphosphate), and the product, together with biotin-labeled cloned hepatitis B virus DNA, was denatured with 0.5M NaOH. This product was hybridized with a probe (not specified) in a microtiter well coated with streptavidin, and the bound complex was detected by incubation with (a) anti-digoxigenin antibody-peroxidase conjugate and (b) ABTS and measurement of the extinction at 405 nm.

L9 ANSWER 8 OF 15 CA COPYRIGHT 1996 ACS DUPLICATE 8
AN 117:63880 CA
TI 'Run-off' polymerization with digoxigenin labelled nucleotides creates highly sensitive and strand specific DNA hybridization probes: synthesis and application
AU Stuerzl, Michael; Oskoui, Kaveh Bastani; Roth, Willi Kurt
CS Dep. Virus Res., Max-Planck-Inst. Biochem., Martinsried, D-8033, Germany
SO Mol. Cell. Probes (1992), 6(2), 107-14
CODEN: MCPRE6; ISSN: 0890-8508
DT Journal
LA English
AB In this paper the in vitro synthesis and application of non-radioactively labeled strand specific DNA probes is described. The probe is labeled by incorporation of nucleotides with the hapten digoxigenin into single-stranded DNA during a 'run-off' reaction catalyzed by Thermus aquaticus (Taq) DNAQ- polymerase. The 'run-off' reaction requires a linearized plasmid template and one primer binding site at a defined distance from the restriction site. Single-stranded DNA can be synthesized during repeated cycles of denaturation, annealing, and extension. The conditions for the incorporation of digoxigenin-11-**dUTP** (dig-11-**dUTP**) during polymn. were optimized to generate strand specific DNA hybridization probes up to a length of 5000 nt. The strand specificity is demonstrated by a dotblot, with in vitro-transcribed target RNA of c-sis. The sensitivity of the probe was tested in a Northern blot, and found to be identical to a probe radiolabeled by nick-translation (specific activity 6.5 .times. 108 cpm .mu.g-1). The resoln. of the signals and speed of development was even superior compared to the radiolabeled probe.

L9 ANSWER 9 OF 15 CA COPYRIGHT 1996 ACS DUPLICATE 9
AN 114:180450 CA
TI Formation of DNA triplexes accounts for arrests of DNA synthesis at d(TC)n and d(GA)n tracts
AU Baran, Nava; Lapidot, Aviva; Manor, Haim
CS Dep. Biol., Technion-Israel Inst. Technol., Haifa, 32000, Israel

SO Proc. Natl. Acad. Sci. U. S. A. (1991), 88(2), 507-11
CODEN: PNASA6; ISSN: 0027-8424
DT Journal
LA English
AB To study the mechanism of arrest of DNA synthesis at d(TC)n and d(GA)n sequences, single-stranded DNA mols. including d(TC)27 or d(TC)31 tracts or a d(GA)27 tract were used as templates for in vitro assays of cDNA synthesis performed by extension of a primer with the Klenow polymerase or the Taq polymerase (Thermus **aquaticus DNA polymerase**). Electrophoresis of the products revealed that arrests occurred around the middle of these tracts. The arrests in the d(TC)n sequences were eliminated when dATP or dGTP was replaced with the analog **7-deaza** dATP or **7-deaza** dGTP, resp., or when the templates were preincubated with the Escherichia coli single-strand binding protein (SSB). Preincubation of the template including a d(GA)27 tract with SSB has also eliminated the arrests at this sequence. Furthermore, arrests did not occur at d[G(**7-deaza** A)]27 of d[(**7-deaza** G)A]27 tracts when mols. including such tracts were used a templates. These results are compatible with the notion that the arrests were caused by formation of d(TC)i.cntdot.d(GA)i.cntdot.d(TC)i and d(GA)i.cntdot.d(GA)i.cntdot.d(TC)i triplexes, in which the bases in the uncopied portions of the d(TC)n tracts, or of the d(GA)27 tract, and the purine bases in the newly synthesized d(TC)i.cntdot.d(GA)i duplexes were bound by hydrogen bonds. In the assays performed with the Taq polymerase, the pH dependence (in the range of 6.0-9.0) and the temp. dependence of the arrests were detd. As the pH was lowered, the arrests in the d(TC)27 tract were enhanced, in line with the expected properties of d(TC)i.cntdot.d(GA)i.cntdot.d(TC)i triplexes. The arrests in the d(GA)27 tract were enhanced by an increase in the pH. At pH 7.2 the arrests in the d(GA)27 tract persisted up to 80.degree., whereas the arrests in the d(TC)27 tract were eliminated at 50.degree.; these results presumably reflect the relative stabilities of the two triplexes mentioned above at this physiol. pH value and could be biol. significant.

L9 ANSWER 10 OF 15 CA COPYRIGHT 1996 ACS DUPLICATE 10
AN 113:207798 CA
TI The use of E. **coli exonuclease III** to generate single stranded DNA in BrdUrd cell-cycle analysis permits simultaneous detection of cell surface antigens
AU Bayer, Jan A.; De Vries, Peter; Herweijer, Hans; Bauman, Jan G. J.
CS Inst. Radiobiol. Immunol., TNO, Rijswijk, Neth.
SO J. Immunol. Methods (1990), 132(1), 13-24
CODEN: JIMMBG; ISSN: 0022-1759
DT Journal
LA English
AB An immunocytochem. method for the simultaneous flow cytometric quantitation of total cellular DNA, incorporated 5-bromo-2'-**deoxyuridine** (BrdUrd) and one or more cell surface antigens has been developed. Biotin labeling of cell surface antigens,

critically tuned fixation techniques, and an enzymic denaturation of cellular DNA are the essential features of this method. Enzymic denaturation of cellular DNA prevented loss of cell surface antigen-bound biotin moieties, and thus preserved cell surface immunofluorescence distribution. After a mild protein extn. and the introduction of breaks into the chromatin using restriction endonucleases, Escherichia **coli exonuclease III** was used to generate stretches of single-stranded DNA. This approach permits detections of the incorporated BrdUrd using anti-BrdUrd monoclonal antibodies. The enzymic denaturation protocol was optimized using in vitro BrdUrd-labeled L1210 murine leukemia cells, and applied to both in vivo and ex vivo BrdUrd-labeled murine bone marrow cells. With this new method it is possible to study DNA content, cell cycle kinetics and cell surface antigen expression simultaneously, and hence functional relationships between these parameters can be investigated.

IT **9037-44-9**, Exodeoxyribonuclease III
RL: ANST (Analytical study)
(cellular DNA denaturation by, for simultaneous detection of cell surface antigens and single-stranded DNA in cell cycle by immunofluorescence)

L9 ANSWER 11 OF 15 CA COPYRIGHT 1996 ACS DUPLICATE 11
AN 112:135591 CA
TI Method for rapid base sequencing of DNA and RNA
IN Jett, James Hubert; Keller, Richard Alan; Martin, John Calvin; Moyzis, Robert Keith; Shera, Edgar Brooks; Stewart, Carleton Colburn; Ratliff, Robert Lafayette
PA United States Dept. of Energy, USA
SO PCT Int. Appl., 23 pp.
CODEN: PIXXD2
PI WO 8903432 A1 890420
DS W: JP, NO
RW: AT, BE, CH, DE, FR, GB, IT, LU, NL, SE
AI WO 88-US3194 880916
PRAI US 87-105375 871007
DT Patent
LA English
AB In the title method, a single fragment of DNA or RNA is provided with identifiable bases and suspended in a moving flow stream, an exonuclease sequentially cleaves individual bases from the end of the suspended fragment, and the moving flow stream maintains the cleaved bases in an orderly train for subsequent detection. Individual bases may be labeled with characteristic fluorescent dyes, and the train of bases excited to fluoresce with the output spectrum characteristic of the individual bases. The process may be automated and can process long (kilobases) strands of DNA or RNA. Poly(A,G)2138 was synthesized using a poly(T)7 primer in a terminal deoxyribonucleotidyl transferase-catalyzed reaction. The complementary strand was then synthesized with biotin-labeled dCTP and biotin-labeled **dUTP**. The biotinylated nucleic acid duplex was sequentially cleaved with exonuclease III at 37.degree. and pH 8. After 2 h, 30% of the DNA was cleaved, and the cleavage

reaction appeared to be still proceeding. Reaction with a nonbiotinylated control DNA yielded 85% cleavage in 2 h. Biotinylation appeared to slow the cleavage reaction using exonuclease III, but the tagged nucleotides were sequentially cleaved from the fragments.

IT **9037-44-9**, Exonuclease III
RL: ANST (Analytical study)
(cleavage of biotinylated DNA with, DNA sequence anal. by exonuclease cleavage in flow stream in relation to)

L9 ANSWER 12 OF 15 CA COPYRIGHT 1996 ACS DUPLICATE 12
AN 110:89890 CA
TI DNA sequencing with Thermus **aquaticus DNA polymerase** and direct sequencing of polymerase chain reaction-amplified DNA
AU Innis, Michael A.; Myambo, Kenneth B.; Gelfand, David H.; Brow, Mary Ann D.
CS Dep. Microb. Genet., Cetus Corp., Emeryville, CA, 94608, USA
SO Proc. Natl. Acad. Sci. U. S. A. (1988), 85(24), 9436-40
CODEN: PNASA6; ISSN: 0027-8424
DT Journal
LA English
AB The highly thermostable DNA polymerase from T. aquaticus (Taq) is ideal for both manual and automated DNA sequencing because it is fast, highly processive, has little or no 3'-exonuclease activity, and is active over a broad range of temps. Sequencing protocols are presented that produce readable extension products >1000 bases having uniform band intensities. A combination of high reaction temps. and the base analog **7-deaza**-2'-deoxyguanosine was used to sequence through G + C-rich DNA and to resolve gel compressions. The polymerase chain reaction (PCR) conditions for direct DNA sequencing of asym. PCR products without intermediate purifn. were modified by using Taq DNA polymerase. The coupling of template prepn. by asym. PCR and direct sequencing should facilitate automation for large-scale sequencing projects.

L9 ANSWER 13 OF 15 CA COPYRIGHT 1996 ACS DUPLICATE 13
AN 100:188438 CA
TI Modified labeled nucleotides and polynucleotides and methods of utilizing and detecting them
IN Engelhardt, Dean; Rabbani, Elazar; Kline, Stanley; Stavrianopoulos, Jannis G.; Kirtikar, Dollie
PA Enzo Biochem, Inc., USA
SO Eur. Pat. Appl., 140 pp.
CODEN: EPXXDW
PI EP 97373 A2 840104
DS R: AT, BE, CH, DE, FR, GB, IT, LI, LU, NL, SE
AI EP 83-106112 830622
PRAI US 82-391440 820623
DT Patent
LA English
AB Nucleotides, polynucleotides, and DNA were chem. modified or labeled with chem. moieties which were readily detectable. These chem.

moieties included carbohydrates and sugars, electron dense substances, magnetic substances, enzymes, coenzymes, hormones, radioactive substances, metals, fluorescent substances, antigens, or antibodies. These chem. modified nucleotides were used for: (1) stimulating or inducing cells to produce lymphokines, cytokinins, and interferon; (2) testing resistance of bacteria to antibiotics; (3) diagnosing genetic disorders, e.g., .beta.-thalassemia; (4) diagnosing tumors; (5) diagnosing bacteria, virus, or fungus infection; and (6) karyotyping chromosomes.

IT **9037-44-9**

RL: ANST (Analytical study)
(digested by, of polydeoxyadenylic acid-polythymidylic acid)

L9 ANSWER 14 OF 15 CA COPYRIGHT 1996 ACS DUPLICATE 14
AN 97:106747 CA
TI Role of exonuclease III in the base excision repair of uracil-containing DNA
AU Taylor, Andrew F.; Weiss, Bernard
CS Sch. Med., Johns Hopkins Univ., Baltimore, MD, 21205, USA
SO J. Bacteriol. (1982), 151(1), 351-7
CODEN: JOBAAY; ISSN: 0021-9193
DT Journal
LA English
AB Mutants of Escherichia coli K-12 deficient in both exonuclease III (the product of the xth gene) and **deoxyuridine** triphosphatase (the dut gene product) are inviable at high temps. and undergo filamentation when grown at such temps. In dut mutants, the **dUTP** pool is greatly enhanced, resulting in an increased substitution of uracil for thymine in DNA during replication. The subsequent removal of uracil from the DNA by uracil-DNA glycosylase produces apyrimidinic sites, at which exonulease III has an endonucleolytic activity. The lethality of dut xth mutants, therefore, indicates that exonuclease III is important for this base-excision pathway and suggests that unrepaired apyrimidinic sites are lethal. The dut xth mutants were viable if they also had a mutation in the uracil-DNA glucosylase (ung) gene; such mutants should not remove uracil from DNA and should not, therefore, generate apyrimidinic sites. In the majority of the temp.-resistant revertants isolated, viability had been restored by a mutation in the dCTP deaminase (dcd) gene; such mutations should decrease **dUTP** prodn. and hence uracil misincorporation. Apparently, in dut mutants, exonuclease III is essential for the repair of uracil-contg. DNA and of apyrimidinic sites.

IT **9037-44-9**

RL: BIOL (Biological study)
(of Escherichia coli, in base excision repair of uracil-contg. DNA)

L9 ANSWER 15 OF 15 CA COPYRIGHT 1996 ACS DUPLICATE 15
AN 91:153410 CA
TI DNA proof-reading by a eukaryotic DNA polymerase
AU Yarranton, G. T.; Banks, G. R.

CS Natl. Inst. Med. Res., London, NW7 1AA, Engl.
SO NATO Adv. Study Inst. Ser., Ser. A (1978), Volume Date 1977, A17(DNA Synth.: Present Future), 479-86
CODEN: NASSDK; ISSN: 0161-0449
DT Journal
LA English
AB The 3' .fwdarw. 5'-exonuclease assocd. with Ustilago maydis DNA polymerase hydrolyzed the noncomplementary dAMP-3H termini in poly(dA).cntdot.(dT)320-dA0.97-3H (I) .apprx.4-fold faster than complementary dTMP-3H in poly(dA).cntdot.(dT)320-dT1.7-3H (II) at 37.degree.; hydrolysis was 12-fold faster at 25.degree.. Similar results were obtained for the hydrolysis of noncomplementary dCMP-3H in poly(dA).cntdot.(dT)320-dC0.82-3H. Thus, the exonuclease shows a preference for noncomplementary 3'-terminal nucleotides which is enhanced at low temps. When II was incubated with enzyme in the presence of dTTP-.alpha.-32P, poly(dT)-32P synthesis was obsd. accompanied by an initial hydrolysis of .apprx.10% of the dTMP-3H, the remainder being resistant. This resistance required substrates (dTTP or **dUTP**) complementary to the template, suggesting a requirement for primer extension by polymerase. However, complete and rapid hydrolysis of dAMP-3H termini in I occurred as poly(dT)-32P synthesis proceeded. Thus, when extension of a complementary 3'-terminus occurs, it is apparently blocked from hydrolysis, whereas extension of a noncomplementary terminus is blocked until complementarity is restored by the exonuclease. The exonuclease is apparently specific for the double-stranded conformation and may bind to double-stranded DNA at a 3'-terminus on 1 strand with the exonuclease site only accommodating 1-3 single-stranded nucleotides. It was concluded that U. maydis DNA polymerase can proof-read in vitro, with the assocd. exonuclease removing noncomplementary primer nucleotides, thus restoring complementarity and providing functional primer termini for the polymerase activity.
IT **71631-66-8**
RL: BIOL (Biological study)
(DNA polymerase-assocd., of Ustilago maydis, DNA proofreading by)

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=> d 1-15 bib abs; fil ca,caplus; taq polymerase# and (l5 or 7(w)deaza? or dutp or d utp or deoxyuridinetriphosphate or deoxyuridine? or deoxy uridine?)

L20 ANSWER 1 OF 15 BIOTECHDS COPYRIGHT 1996 DERWENT INFORMATION LTD
AN 94-11411 BIOTECHDS
TI Single-buffer combined reverse transcription and DNA amplification by a Thermus **thermophilus DNA-polymerase;**
thermostable DNA-polymerase with reverse-transcriptase activity application in simultaneous polymerase chain reaction and reverse transcription (conference abstract)
AU Sigua C L; Gelfand D H; Myers T W
CS Roche-Mol.Syst.
LO Roche Molecular Systems, 1145 Atlantic Avenue, Alameda, CA 94501, USA.
SO Abstr.Gen.Meet.Am.Soc.Microbiol.; (1994) 94 Meet., 551
CODEN: 0005P

DT Journal
LA English
AN 94-11411 BIOTECHDS
AB A recombinant DNA-polymerase (EC-2.7.7.7) from Thermus thermophilus (rTth pol) possesses efficient reverse-transcriptase (EC-2.7.7.49) activity in the presence of Mn2+. This may facilitate use of a single enzyme for reverse transcription (RT) and polymerase chain reaction (PCR), allow for increased primer binding specificity and alleviate the secondary structures present in the RNA template. Reaction conditions compatible with rTth pol performing both RT and PCR in a buffer containing Mn2+ were determined. The Mn2+ concentration optimum was different for RNA and DNA templates and the reaction was very sensitive to the free Mn2+ concentration unless a metal buffer such as bicine was used. Detection of a specific mRNA from 80 pg of total cellular RNA or 100 copies of synthetic cRNA was possible by standard analysis. The speed, sensitivity, robustness, and the ability to incorporate **dUTP** for carryover prevention by uracil-N-glycosylase achieved by using rTth pol for RT/PCR further extended their ability to detect cellular and viral RNA. Coupling of this assay with a quantitative detection system would make disease monitoring feasible. (0 ref)

L20 ANSWER 2 OF 15 EMBASE COPYRIGHT 1996 ELSEVIER SCI. B.V.
AN 95139437 EMBASE
TI Sister chromatid differentiation after in situ detection of ultraviolet-induced DNA breaks under electron microscopy.
AU Fernandez J.L.; Campos A.; Goyanes V.; Buno I.; Gosalvez J.
CS Centro Oncologico de Galicia, Lab Dosimetria Biologica-Genetica, Avda de Montserrat s/n, 15006 La Coruna, Spain
SO Biology of the Cell, (1994) 82/1 (33-37).
ISSN: 0248-4900 CODEN: BCELDF
CY France
DT Journal
FS 022 Human Genetics
029 Clinical Biochemistry
LA English
SL English
AB Chinese hamster DON cells with 5-bromodeoxyuridine (BrdU)-substituted chromosomes were ultraviolet (UV)-exposed and processed for in situ detection of induced DNA breaks under electron microscopy. For this purpose, UV-induced breaks were amplified by an exonuclease III digestion to obtain single stranded DNA motifs which could hybridize with oligonucleotides of random sequences. These reannealed motifs could be used as primers which were extended by the Klenow polymerase, incorporating biotinylated-**dUTP** that was detected by a gold-tagged streptavidin. After processing, the chromatid whose DNA was BrdU-substituted in one strand showed a higher electron density than the chromatid substituted in both strands. In contrast, the unifilarly substituted chromatid showed about twice the labelling of DNA breaks as the bifilarly substituted one. This result could be the consequence of a greater loss of chromatin tracts in the bifilarly substituted chromatid, as implied

by an X-ray microanalysis which showed that the amount of phosphorous lost by the bifilarly substituted chromatid was higher than that of the unifilarly substituted chromatid.

L20 ANSWER 3 OF 15 BIOSIS COPYRIGHT 1996 BIOSIS
AN 94:60901 BIOSIS
DN 97073901
TI Selective digestion of mouse chromosomes with restriction endonucleases: Oligonucleotide priming of single-stranded DNA produced with exonuclease III.
AU Gosalvez J; Lopez-Fernandez C; De La Vega C G; Mezzanotte R; Fernandez J L; Goyanes V
CS Dep. Biol. Edifico de Biol., Fac. de Ciencias, Universidad Autonoma de Madrid, 28049 Madrid, SPA
SO Genome 36 (2). 1993. 230-234. ISSN: 0831-2796
LA English
AB L-929 mouse chromosomes prepared for electron microscopy have been treated with MspI, EcoRI, and HaeIII restriction endonucleases (REs). RE-induced nicks were amplified with exonuclease III to obtain single-stranded DNA (ss-DNA) motifs. The ss-DNA produced was enough to permit hybridization of a series of random oligonucleotides. These can be used as primers, which are extended by the Klenow fragment using non-isotopic labelled **dUTP**. The incorporation of biotinylated **dUTP** is detected with a gold-tagged streptoavidin as the reporter molecule. This allows, in mouse chromosomes, the detection of different rates of sensitivity to the digestion with specific REs in distinct intraheterochromatic DNA subsets. In addition, these results show that enzymatic production of ss-DNA seems to be adequate for electron microscopy work since the chromatin fiber is preserved better than in denatured DNA produced with heat, NaOH, or formamide.

L20 ANSWER 4 OF 15 BIOSIS COPYRIGHT 1996 BIOSIS
AN 93:317417 BIOSIS
DN BA96:25767
TI DETECTION OF DNA STRAND BREAKS INDUCED BY HYDROXYL RADICALS IN NUCLEAR AND CHROMOSOMAL CHROMATIN BY ELECTRON MICROSCOPY.
AU FERNANDEZ J L; GOSALVEZ J; GOYANES V J
CS LAB. DOSIMETRIA BIOL., CENT. ONCOL. DE GALICIA, AVDA DE MONTSERRAT S/N, 150006 LA CORUNA, SPAIN.
SO CYTOBIOS 73 (294-295). 1993. 189-195. CODEN: CYTBAI ISSN: 0011-4529
LA English
AB Chinese hamster Don cells were treated with 10 mM hydrogen peroxide. DNA strand breaks induced by hydroxyl radicals were amplified in 3' termini by an exonuclease III digestion, resulting in single stranded DNA motifs. In situ detection of these motifs was performed on chromatin fibres of isolated whole-mounted nuclei and chromosomes by a random priming procedure, using biotinylated-**dUTP** which bound a gold-tagged streptavidin. This approach facilitated the location of hydroxyl radical induced DNA breaks, specifically on 20-30 nm diameter chromatin fibres, by transmission electron microscopy.

L20 ANSWER 5 OF 15 BIOSIS COPYRIGHT 1996 BIOSIS DUPLICATE 1
AN 93:319748 BIOSIS
DN BA96:28098
TI LOW INCORPORATION OF DUMP BY SOME THERMOSTABLE DNA POLYMERASES MAY LIMIT THEIR USE IN PCR AMPLIFICATIONS.
AU SLUPPHAUG G; ALSETH I; EFTEDAL I; VOLDEN G; KROKAN H E
CS UNIGEN CENT. MOL. BIOL., UNIV. TRONDHEIM, N-7005 TRONDHEIM, NORWAY.
SO ANAL BIOCHEM 211 (1). 1993. 164-169. CODEN: ANBCA2 ISSN: 0003-2697
LA English
AB Incorporation of dUMP instead of dTMP is frequently used to control carryover contamination during PCR amplifications. We have tested four thermostable DNA polymerases for their ability to utilize **dUTP** as a substrate in PCR. Amplification of products in the presence of **dUTP** instead of dTTP was good with Thermus **aquaticus DNA polymerase** but highly inefficient with three other thermostable DNA polymerases. The latter was due to: (a) lower incorporation of dUMP relative to dTMP, (b) increased proofreading toward dUMP in DNA, (c) relative termination at dUMP residues as verified by sequencing reactions in the presence of **dUTP**, (d) thermostable dUTPase activity in the commercial enzyme preparation. The last point only applies to Pyrococcus furiosus DNA polymerase. This study demonstrates that various thermostable DNA polymerases utilize dTTP and **dUTP** with highly different efficiencies and thus the choice of DNA polymerase may be critical for amplification of DNA.

L20 ANSWER 6 OF 15 EMBASE COPYRIGHT 1996 ELSEVIER SCI. B.V.
AN 92211618 EMBASE
TI Characterization of biotinylated repair regions in reversibly permeabilized human fibroblasts.
AU Huijzer J.C.; Smerdon M.J.
CS Dept. of Biochemistry and Biophysics, Washington State University, Pullman, WA 99164-4660, United States
SO BIOCHEMISTRY, (1992) 31/21 (5077-5084).
ISSN: 0006-2960 CODEN: BICHAW
CY United States
DT Journal
FS 029 Clinical Biochemistry
LA English
SL English
AB We have examined the incorporation of biotinyl-11-**deoxyuridine** triphosphate (BiodUTP) into excision repair patches of UV-irradiated confluent human fibroblasts. Cells were reversibly permeabilized to BiodUTP with lysolecithin, and biotin was detected in DNA on nylon filters using a streptavidin/alkaline phosphatase colorimetric assay. Following a UV dose of 12 J/m2, maximum incorporation of BiodUTP occurred at a lysolecithin concentration (80-100 .mu.g/mL) similar to that for incorporation of dTTP. Incorporation of BiodUTP into repair patches increased with UV dose up to 4 and 8 J/m2 in two normal human fibroblast strains, while no incorporation of BiodUTP was observed in xeroderma pigmentosum (group A) human fibroblasts. The repair-incorporated biotin was not removed from the DNA over a 48-h period, and only

slowly disappeared after longer times (.apprx.30% in 72 h), while little of the biotin remained in cells induced to divide. Furthermore, the stability of the biotin in repaired DNA was unaffected by a second dose of UV radiation several hours after the biotin-labeling period to induce a 'second round' of excision repair. Exonuclease III digestion and gap-filling with DNA polymerase I indicate that the majority of biotin-labeled repair patches (.apprx.80%) are rapidly ligated in confluent human cells. However, the remaining patches were not ligated after a 24-h chase period, in contrast to dTTP- labeled repair patches. The BiodUMP repair label in both chromatin and DNA is preferentially digested by staphylococcal nuclease, preventing the use of this enzyme for nucleosome mapping in these regions. However, restriction enzyme and DNase I digestions of the isolated nuclei demonstrate that at least some of the repair-incorporated BiodUMP becomes associated with nucleosome core DNA following nucleosome rearrangement. Therefore, the biotin tag does not appear to prevent the folding of nascent repair patches into native nucleosome structures.

L20 ANSWER 7 OF 15 BIOSIS COPYRIGHT 1996 BIOSIS DUPLICATE 2
AN 91:158854 BIOSIS
DN BA91:84654
TI FORMATION OF DNA TRIPLEXES ACCOUNTS FOR ARRESTS OF DNA SYNTHESIS AT DTC-N AND DGA-N TRACTS.
AU BARAN N; LAPIDOT A; MANOR H
CS DEP. BIOL., TECHNION-ISRAEL INST. TECHNOL., HAIFA 32000, ISRAEL.
SO PROC NATL ACAD SCI U S A 88 (2). 1991. 507-511. CODEN: PNASA6 ISSN: 0027-8424
LA English
AB To study the mechanism of arrest of DNA synthesis at d(TC)n and d(GA)n sequences, single-stranded DNA molecules including d(TC)27 or d(TC)31 tracts or a d(GA)27 tract were used as templates for in vitro assays of complementary DNA synthesis performed by extension of a primer with the Klenow polymerase or the Taq polymerase (Thermus **aquaticus DNA polymerase**).
Electrophoresis of the products revealed that arrests occurred around the middle of these tracts. The arrests in the d(TC)n sequences were eliminated when dATP or dGTP was replaced with the analogue **7 -deaza** dATP or **7-deaza** dGTP,
respectively, or when the templates were preinucbated with the Escherichia coli single-strand binding protein (SSB). Preincubation of the template including a d(GA)27 tract with SSB has also eliminated the arrests at this sequence. Furthermore, arrests did not occur at d[G(**7-deaza** A)]27 or d[(**7-deaza** G)A]27 tracts when molecules including such tracts were used as templates. These results are compatible with the notion that the arrests were caused by formation of d(TC)i .cntdot. d(GA)i .cntdot. d(TC)i and d(GA)i .cntdot. d(GA)i .cntdot. d(TC)i triplexes, in which the bases in the uncopied portions of the d(TC)n tracts, or of the d(GA)27 tract, and the purine bases in the newly synthesized d(TC)i .cntdot. d(GA)i duplexes were bound by hydrogen bonds. In the assays performed with the Taq polymerase, the pH dependence (in the range of 6.0-9.0) and the temperature dependence of the arrests were

determined. As the pH was lowered, the arrests in the d(TC)27 tract were enhanced, in line with the expected properties of d(TC)i .cntdot. d(GA)i .cntdot. d(TC)i triplexes. The arrests in the d(GA)27 tract were enhanced by an increase in the pH. At pH 7.2 the arrests in the d(GA)27 tract persisted up to 80.degree. C, whereas the arrests in the d(TC)27 tract were eliminated at 50.degree. C; these results presumably reflect the relative stabilities of the two triplexes mentioned above at this physiological pH value and could be biologically significant.

L20 ANSWER 8 OF 15 BIOTECHDS COPYRIGHT 1996 DERWENT INFORMATION LTD
AN 90-08045 BIOTECHDS
TI Structure-independent DNA amplification; using polymerase chain reaction with incorporation of **7-deaza**-2'-deoxyguanosine-5'-triphosphate
PA Cetus
PI WO 9003443 5 Apr 1990
AI WO 89-US4100 19 Sep 1989
PRAI US 88-248556 23 Sep 1988
DT Patent
LA English
OS WPI: 90-132279 [17]
AN 90-08045 BIOTECHDS
AB A method for structure-independent DNA amplification using the polymerase chain reaction (PCR) is new. It comprises: (1) hybridizing DNA with a pair of oligonucleotide primers, an agent for polymerization, dATP, dCTP, TTP and c7dGTP to form an extension product, which can function as a template; (2) separating the extension product from the templates; and (3) repeating steps (1) and (2) using the extension product formed in step (2). The dGTP is also present in step (1) and the agent for polymerization is preferably a thermostable DNA-polymerase (EC-2.7.7.7), especially Thermus **aquaticus DNA-polymerase**.
The DNA is prepared from RNA by synthesizing cDNA from RNA and making the cDNA double stranded. The concentration of dATP, TTP, dCTP, dGTP and c7dGTP is preferably 10 uM-1.5 mM. The ratio of dGTP and c7dGTP is preferably 1:3 and the dGTP is preferably used at 50 uM. The c7dGTP is preferably incorporated in cDNA and is double-stranded. The incorporation of **7-deazaguanine** into the amplified DNA results in an increase in specificity of PCR on templates that contain secondary structure and/or compressed regions. (16pp)

L20 ANSWER 9 OF 15 WPIDS COPYRIGHT 1996 DERWENT INFORMATION LTD
AN 90-132278 [17] WPIDS
DNC C90-058134
TI Nucleotide sequence determn. by chain termination - using DNA polymerase from Thermus aquaticus to catalyse primer extension reactions.
DC B04 D16
IN BROW, M A D; GELFAND, D H; INNIS, M A; MYAMBO, K B; BROW, M A
PA (HOFF) HOFFMANN LA ROCHE & CO AG F; (CETU) CETUS CORP; (HOFF) HOFFMANN-LA ROCHE AG F

CYC 15
PI WO 9003442 A 900405 (9017)*
RW: AT BE CH DE FR GB IT LU NL SE
W: AU JP
AU 8943003 A 900418 (9027)
EP 437459 A 910724 (9130)
R: AT BE CH DE FR GB IT LI LU NL SE
US 5075216 A 911224 (9203)
JP 04501205 W 920305 (9216) 11 pp
AU 647015 B 940317 (9416)
CA 1332561 C 941018 (9442)
ADT EP 437459 A EP 89-910787 890919; US 5075216 A US 88-249367 880923; JP 04501205 W JP 89-510171 890919; AU 647015 B AU 89-43003 890919; CA 1332561 C CA 89-612500 890922
FDT JP 04501205 W Based on WO 903442; AU 647015 B Previous Publ. AU 8943003, Based on WO 9003442
PRAI US 88-249367 880923
AN 90-132278 [17] WPIDS
AB WO 9003442 A UPAB: 940223
A method for determining a nucleotide sequence for a nucleic acid segment by a dideoxynucleotide-5'-triphosphate chain termination procedure is claimed in which the sequence is detd. by extending an oligonucleotide primer in a template-dependent manner in the presence of Thermus aquaticus (Taq) DNA polymerase, 4 deoxyribonucleotide -5'-triphosphates (dNTPs) and a dideoxynucleotide -5'-triphosphate (ddNTP).
ADVANTAGE - The ability of Taq DNA polymerase to operate at high temp. and low salt allows heat-destabilisation of hairpins during the sequencing reaction, permitting the enzyme to read through such structures. The concomitant use of a structure-destabilising dGTP analogue such as 7-**deaza**-2'-deoxyguanosine -5'-triphosphate (c7dGTP) yields sequencing prods. from such difficult to sequence DNA that were fully resolved upon electrophoresis. The method provides an absence of background hands and uniform intensity of the radioactive fragments. The Taq DNA polymerase is very processive and has very little if any proofreading activity. @(30pp Dwg.No.0/3)
0/3
ABEQ US 5075216 A UPAB: 930928
Determn. of a nucleotide sequence in a nucleic acid segment by a dideoxynucleoside-5'-triphosphate chain termination method comprises extending an oligonucleotide primer according to a template, in the presence of Thermus **aquaticus DNA polymerase**, four deoxynucleoside-5'-triphosphate (ddATP, ddCTP, ddGTP or ddTTP); and a dideoxynucleoside-5'-triphosphate (ddATP, ddCTP, ddGTP or ddTTP); and electrophoretic analysis of the prods..
USE - The process is applicable to molecular biology, genetics, diagnosis and forensic science.

L20 ANSWER 10 OF 15 BIOSIS COPYRIGHT 1996 BIOSIS DUPLICATE 4
AN 90:472009 BIOSIS
DN BA90:111429

TI THE USE OF ESCHERICHIA-**COLI EXONUCLEASE III** TO GENERATE SINGLE STRANDED DNA IN BRDURD CELL-CYCLE ANALYSIS PERMITS SIMULTANEOUS DETECTION OF CELL SURFACE ANTIGENS.

AU BAYER J A; DE VRIES P; HERWEIJER H; BAUMAN J G J

CS TNO INST. APPLIED RADIOBIOL. IMMUNOL., DEP. CELL BIOL., CYTOMETRY SECT., P.O. BOX 5815, 2280 HV RIJSWIJK, NETHERLANDS.

SO J IMMUNOL METHODS 132 (1). 1990. 13-24. CODEN: JIMMBG ISSN: 0022-1759

LA English

AB An immunocytochemical method for the simultaneous flow cytometric quantitation of total cellular DNA, incorporated 5-bromo-2'-**deoxyuridine** (.BETA.rdUrd) and one or more cell surface antigens has been developed. Biotin labeling of cell surface antigens, critically tuned fixation techniques and an enzymatic denaturation of cellular DNA are the essential features of this method. Enzymatic denaturation of cellular DNA was shown to prevent loss of cell surface antigen-bound biotin moieties, and thus to preserve cell surface immunofluorescence distribution. After a mild protein extraction and the introduction of breaks into the chromatin using restriction endonucleases, E. **coli exonuclease III** was used to generate stretches of single stranded DNA. This approach permits detection of the incorporated BrdUrd using anti-BrdUrd monoclonal antibodies. The enzymatic denaturation protocol was optimized using in vitro BrdUrd-labeled L1210 murine leukemia cells, and applied to both in vivo and ex vivo BrdUrd-labeled murine bone marrow cells. With this new method it is possible to study DNA content, cell cycle kinetics and cell surface antigen expression simultaneously, and hence functional relationships between these parameters can be investigated.

L20 ANSWER 11 OF 15 BIOTECHDS COPYRIGHT 1996 DERWENT INFORMATION LTD

AN 89-01756 BIOTECHDS

TI DNA sequencing using Taq polymerase; new method with Thermus **aquaticus DNA-polymerase**

AU Peterson M G

LO The Walter and Eliza Hall Institute of Medical Research, PO Royal Melbourne Hospital, Victoria 3050, Australia.

SO Nucleic Acids Res.; (1988) 16, 22, 10915 CODEN: NARHAD

DT Journal

LA English

AN 89-01756 BIOTECHDS

AB A new method was developed using thermostable DNA-polymerase (EC-2.7.7.7) of Thermus aquaticus (Taq) in DNA sequencing incorporating **7-deaza**-GTP at 70 deg, eliminating inhibitory effects of DNA template secondary structure. For the labeling reaction, phage M13 primer was annealed to the phage M13 template and extended in the presence of labeled dNTPs. For the termination reaction, the product was divided into aliquots with appropriate deoxy/dideoxynucleotide mixes. The single-stranded phage M13 template used to test this method was a

cDNA fragment encoding lichenase (EC-3.2.1.73) from wheat (Triticum aestivum), containing a region with strong secondary structure that could not be unambiguously sequenced on either strand using sequenase. With sequenase at 37 deg, strong stops occurred in all 4 tracks at 1 position. Sequencing the template with Taq DNA-polymerase at 70 deg gave an unambiguous reading. Taq DNA-polymerase has further advantages over sequenase: it does not require a separate annealing reaction, is more stable, allowing all labeling constituents (except the template) to be mixed before aliquoting, and is much cheaper. (1 ref)

L20 ANSWER 12 OF 15 BIOSIS COPYRIGHT 1996 BIOSIS DUPLICATE 5
AN 89:123948 BIOSIS
DN BA87:58601
TI DNA SEQUENCING WITH THERMUS-**AQUATICUS DNA POLYMERASE** AND DIRECT SEQUENCING OF POLYMERASE CHAIN REACTION-AMPLIFIED DNA.
AU INNIS M A; MYAMBO K B; GELFAND D H; BROW M A D
CS DEP. MICROBIAL GENETICS, CETUS CORP., 1400 FIFTY-THIRD ST., EMERYVILLE, CA 94608.
SO PROC NATL ACAD SCI U S A 85 (24). 1988. 9436-9440. CODEN: PNASA6 ISSN: 0027-8424
LA English
AB The highly thermostable DNA polymerase from Thermus aquaticus (Taq) is ideal for both manual and automated DNA sequencing because it is fast, highly processive, has little or no 3'-exonuclease activity, and is active over a broad range of temperatures. Sequencing protocols are presented that produce readable extension products > 1000 bases having uniform band intensities. A combination of high reaction temperatures and the base analog **7-deaza** -2'-deoxyguanosine was used to sequence through G+C-rich DNA and to resolve gel compressions. We modified the polymerase chain reaction (PCR) conditions for direct DNA sequencing of asymmetric PCR products without intermediate purification by using Taq DNA polymerase. The coupling of template preparation by asymmetric PCR and direct sequencing should facilitate automation for large-scale sequencing projects.

L20 ANSWER 13 OF 15 BIOTECHDS COPYRIGHT 1996 DERWENT INFORMATION LTD
AN 88-11096 BIOTECHDS
TI Rapid production of vector-free biotinylated probes using the polymerase chain reaction;
construction of amplified biotin-labeled DNA probe
AU Lo Y M D; Mehal W Z; Fleming K A
LO University of Oxford, Nuffield Department of Pathology, John Radcliffe Hospital, Oxford, OX3 9DU, UK.
SO Nucleic Acids Res.; (1988) 16, 17, 8719
CODEN: NARHAD
DT Journal
LA English
AN 88-11096 BIOTECHDS
AB Biotin-labeled vector-free DNA probes were produced by a polymerase chain reacton (PCR), with 150 mM dTTP and 50 uM biotinylated

dUTP (bio-**dUTP**). The target was 1 ng plasmid pHBV130 with a full length hepatitis-B virus insert. 5' And 3' primers were obtained from known sequences. 25 Cycles (2 min at 94 deg and 55 deg, 3 min at 72) were carried out. When the labeled product was used as a hybridization probe, 1 pg target DNA could be detected. As an alternative method, after amplification without bio-**dUTP** the mixture was diluted, denatured and 10-50 uM bio-**dUTP** was added, with labeling at 24, 37, 55 or 72 deg with or without additional Thermus **aquaticus DNA -polymerase** (EC-2.7.7.7). However, the first protocol was faster and more economical. To avoid non-specific labeling the amount of plasmid DNA was kept to 1 ng or below. The method was very specific and there was little hybridization to the vector band. 5-10 ug probe was synthesized in less than 4 hr. The method could be used generally for target sequences below 2.5 kb. A probe for detection of amplified product could be produced using primers internal to the target sequence. (4 ref)

L20 ANSWER 14 OF 15 BIOTECHDS COPYRIGHT 1996 DERWENT INFORMATION LTD
AN 89-03099 BIOTECHDS
TI DNA sequencing with Thermus **aquaticus DNA-polymerase** and direct sequencing of polymerase chain reaction-amplified DNA;
DNA amplification
AU Innis M A; Myambo K B; Gelfand D H; Brow M A D
CS Cetus
LO Department of Microbial Genetics, Cetus Corporation, 1400 Fifty-Third Street, Emeryville, CA 94608, USA.
SO Proc.Natl.Acad.Sci.U.S.A.; (1988) 85, 24, 2436-40
CODEN: PNASA6
DT Journal
LA English
AN 89-03099 BIOTECHDS
AB The highly thermostable DNA-polymerase (EC-2.7.7.7) from Thermus aquaticus (Taq) is suitable for manual and automated DNA sequencing because it is rapid, has little or no 3'-exonuclease activity, and is active over a broad temp. range. DNA sequencing protocols were developed to produce readable extension products of over 1000 bases, having uniform band intensities. A combination of high reaction temp. and the base analog **7-deaza** -2'-deoxyguanosine was used to sequence G+C-rich DNA and to resolve gel compressions. The enzyme worked equally well with either 5'-labeled primers or by incorporation of label in a 2-step reaction protocol. Both approaches generated sequencing ladders free of background bands which were uniform and readable over long distances. The polymerase chain reaction conditions were modified for direct DNA sequencing of asymmetric polymerase chain reaction products without intermediate purification by using Taq DNA-polymerase. The coupling of template preparation by asymmetric polymerase chain reaction and direct sequencing should facilitate automation for large-scale DNA sequencing projects. (19 ref)

L20 ANSWER 15 OF 15 BIOSIS COPYRIGHT 1996 BIOSIS

AN 83:191449 BIOSIS
DN BA75:41449
TI ROLE OF EXO NUCLEASE III IN THE BASE EXCISION REPAIR OF URACIL CONTAINING DNA.
AU TAYLOR A F; WEISS B
CS DEP. MOLECULAR BIOL. AND GENETICS, JOHN HOPKINS UNIV. SCH. MED., BALTIMORE, MD 21205.
SO J BACTERIOL 151 (1). 1982. 351-357. CODEN: JOBAAY ISSN: 0021-9193
LA English
AB Mutants of Escherichia coli K-12 deficient in both exonuclease III (the product of the xth gene) and **deoxyuridine** triphosphatase (the dut gene product) are inviable at high temperatures and undergo filamentation when grown at such temperatures. In dut mutants, the **dUTP** pool is known to be greatly enhanced, resulting in an increased substitution of uracil for thymine in DNA during replication. The subsequent removal of uracil from the DNA by uracil-DNA glycosylase produces apyrimidinic sites, at which exonuclease III is known to have an endonucleolytic activity. The lethality of dut xth mutants, therefore, indicates that exonuclease III is important for this base-excision pathway and suggests that unrepaired apyrimidinic sites are lethal. dut xth Mutants were viable if they also had a mutation in the uracil-DNA glycosylase (ung) gene; such mutants should not remove uracil from DNA and should not, therefore, generate apyrimidinic sites. In the majority of the temperature-resistant revertants isolated, viability had been restored by a mutation in the dCTP deaminase (dcd) gene; such mutations should decrease **dUTP** production and hence uracil misincorporation. In dut mutants, exonuclease III is essential for the repair of uracil-containing DNA and of apyrimidinic sites.

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=> s taq polymerase# and (l5 or 7(w)deaza? or dutp or d utp or deoxyuridinetriphosphate or deoxyuridine? or deoxy uridine?)
L21 16 FILE CA
L22 16 FILE CAPLUS

TOTAL FOR ALL FILES
L23 32 TAQ POLYMERASE# AND (L5 OR 7(W) DEAZA? OR DUTP OR D UTP OR DEOXYURIDINETRIPHOSPHATE OR DEOXYURIDINE? OR DEOXY URIDIN

E?)

=> s l23 not l9
L#S CREATED BY SORT OR DUPLICATE ARE NOT ALLOWED
An L# created by a SORT or a DUPLICATE command cannot be used with the SEARCH command. Enter "DISPLAY HISTORY" at the arrow prompt (=>) to determine the original L#.

=> s l23 not l8
L24 14 FILE CA
L25 14 FILE CAPLUS

TOTAL FOR ALL FILES
L26 28 L23 NOT L8

=> dup rem l26
PROCESSING COMPLETED FOR L26
L36 14 DUP REM L26 (14 DUPLICATES REMOVED)

=> d his l21-l26; d l36 1-14 .bevstr; fil
biosi,medl,embas,lifesci,biotechds,wpids,confsci,dissabs,scisearch

(FILE 'CA, CAPLUS' ENTERED AT 16:48:32 ON 08 MAY 96)
L21 16 FILE CA
L22 16 FILE CAPLUS
TOTAL FOR ALL FILES
L23 32 S TAQ POLYMERASE# AND (L5 OR 7(W)DEAZA? OR DUTP OR D UTP
L24 14 FILE CA
L25 14 FILE CAPLUS
TOTAL FOR ALL FILES
L26 28 S L23 NOT L8

L36 ANSWER 1 OF 14 CA COPYRIGHT 1996 ACS DUPLICATE 1
AN 122:124149 CA
TI a more reliable PCR for detection of Mycobacterium tuberculosis in clinical samples
AU Kox, L. F. F.; Rhienthong, D.; Miranda, A. Medo; Udomsantisuk, N.; Ellis, K.; van Leeuwen, J.; van Heusden, S.; Kuijper, S.; Kolk, A. H. J.
CS R. Trop. Inst., Acad. Med. Cent., Amsterdam, Neth.
SO J. Clin. Microbiol. (1994), 32(3), 672-8
CODEN: JCMIDW; ISSN: 0095-1137
DT Journal
LA English
AB Diagnostic techniques based on PCR have two major problems: false-pos. reactions due to contamination with DNA fragments from previous PCRs (amplicons) and false-neg. reactions caused by inhibitors that interfere with the PCR. We have improved our previously reported PCR based on the amplification of a fragment of the Mycobacterium tuberculosis complex-specific insertion element IS6110 with respect to both problems. False-pos. reactions caused

by amplicon contamination were prevented by the use of uracil-N-glycosylase and **dUTP** instead of dTTP. We selected a new set of primers outside the region spanned by the formerly used primers to avoid false-pos. reactions caused by dTTP-contg. amplicons still present in the lab. With this new primer set, 16 copies of the IS6110 insertion element, the equiv. of two bacteria, could be amplified 1010 times in 40 cycles, resulting in a mean efficiency of 77% per cycle. To detect the presence of inhibitors of the **Taq polymerase**, which may cause false-neg. reactions, part of each sample was spiked with M. tuberculosis DNA. The DNA purifn. method using guanidinium thiocyanate and diatoms effectively removed most or all inhibitors of the PCR. However, this was not suitable for blood samples, for which we developed a proteinase K treatment followed by phenol-chloroform extn. This method permitted detection of 20 M. tuberculosis bacteria per mL of whole blood. Various lab. procedures were introduced to reduce failure or inhibition of PCR and avoid DNA cross contamination. We have tested 218 different clin. specimens obtained from patients suspected of having tuberculosis. The samples included sputum (n = 145), tissue biopsy samples (n = 25), cerebrospinal fluid (n = 15), blood (n = 14), pleural fluid (n = 9), feces (n = 7), fluid from fistulae (n = 2), and pus from a wound (n = 1). The results obtained by PCR were consistent with those obtained with culture, which is the "gold std.". We demonstrate that PCR is a useful technique for the rapid diagnosis of tuberculosis at various sites.

IT **1173-82-6, DUTP**
RL: ARG (Analytical reagent use); BUU (Biological use, unclassified); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(in PCR reactions for control of false-positives; a more reliable PCR for detection of Mycobacterium tuberculosis in clin. samples)

L36 ANSWER 2 OF 14 CA COPYRIGHT 1996 ACS DUPLICATE 2
AN 120:155013 CA
TI Production of hybridization probes by the PCR utilizing digoxigenin-modified nucleotides
AU McCreery, Tom; Helentjaris, Tim
CS Dep. Plant Sci., Univ. Arizona, Tucson, AZ, USA
SO Methods Mol. Biol. (Totowa, N. J.) (1994), 28(Protocols for Nucleic Acid Analysis by Nonradioactive Probes), 67-71
CODEN: MMBIED; ISSN: 1064-3745
DT Journal
LA English
AB A method for the prepn. of digoxigenin-labeled DNA probes is described. Digoxigenin-11-**dUTP** is a good substrate for the **Taq polymerase** making PCR a prefered method for the prepn. of hybridization probes.

L36 ANSWER 3 OF 14 CA COPYRIGHT 1996 ACS DUPLICATE 3
AN 123:1988 CA
TI Polymerase chain reaction-based diagnostic assay to detect cattle chronically infected with Babesia bovis

AU Figueroa, J. V.; Chieves, L. P.; Johnson, G. S.; Goff, W. L.; Buening, G. M.
CS Dep. Veterinary Microbiol., UMC, Columbia, MO, 65211, USA
SO Rev. Latinoam. Microbiol. (1994), 36(1), 47-55
CODEN: RLMIAA; ISSN: 0034-9771
DT Journal
LA English
AB From a B. bovis gene sequence coding for a 60 kDa merozoite surface protein previously published, two sets of primers were designed for the Polymerase Chain Reaction (PCR) assay. Primer set BoF/BoR was used to prime **Taq Polymerase** DNA amplification of a 350 bp fragment of the target B. bovis DNA. Primer set BoFN/BoRN was used to prep. a PCR-synthesized, Digoxigenin-**dUTP**-labeled probe (291 bp) which would hybridize to a sequence within the PCr-amplified parasite target DNA. PCR amplification of target DNA obtained from in vitro-cultured B. bovis and nucleic acid hybridization of amplified product with the nonradioactive DNA probe showed that a 350 bp fragment could be detected when as little as 10 pg of genomic parasite DNA was utilized in the assay. A fragment of similar size was amplifed from genomic DNA from four other B. bovis isolates but not from B. bigemina, Anaplasma marginale, or bovine leukocyte DNA. The PCR product was detected in blood samples contg. approx. 3 B. bovis-infected erythrocytes (20 .mu.L of packed cells with a parasitemia of 0.000001%). By using the PCR/DNA probe assay, 16 out of 20 animals exptl. inoculated with B. bovis were detected pos., whereas no PCR product was obsd. in bovine blood samples collected from 20 B. bigemina-infected, and 20 uninfected cattle tested. The PCR-DNA probe assay was shown to be sensitive in detecting some cattle with B. bovis-chronic infection. The specificity and high anal. sensitivity of the test provides a valuable tool to apply in conducting epidemiol. studies.

L36 ANSWER 4 OF 14 CA COPYRIGHT 1996 ACS DUPLICATE 4
AN 118:185109 CA
TI Novel amplification method for polynucleotide assays
IN Dattagupta, Nanibhushan; Sullivan, Elizabeth C.
PA Miles Inc., USA
SO Eur. Pat. Appl., 7 pp.
CODEN: EPXXDW
PI EP 530526 A1 930310
DS R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE
AI EP 92-113394 920806
PRAI US 91-744548 910813
DT Patent
LA English
AB A nucleic acid sequence is detected in a sample by (1) treating the sample under hybridization conditions with an oligonucleotide that lacks a recognition site for enzyme digestion, (2) extending the hybridization product by adding polymerase and nucleoside triphosphates to create, on the oligonucleotide strand, a recognition site for enzyme digestion, (3) hybridizing the

oligonucleotide strand to a labeled probe which is immobilized or immobilizable and contains a recognition site for enzyme digestion that is completely or partially complementary to the recognition site for enzyme digestion on the oligonucleotide strand, (4) digesting the hybridization product with restriction endonuclease, and (5) detecting the sepd. label which is released in soln. A kit for detection of a nucleic acid sequence comprises a labeled probe, an oligonucleotide sequence for extension, and a restriction endonuclease. Thus, Chlamydia DNA was detected by use of a synthetic 22-mer oligonucleotide representing a portion of the gene for the major outer membrane protein of C. trachomatis, which was 5' end labeled with 32P using T4 polynucleotide kinase and 3' end labeled with biotin by thermocycling 30 times in the presence of biotin-11-**dUTP**, **Taq polymerase**, and a DNA sample. The amplified product was immobilized on streptavidin-coated magnetic particles, hybridized with a single-stranded complementary oligonucleotide contg. a restriction site for AluI, digested with AluI, and released radioactivity was detd. in the supernatant, after magnetic particle sepn., by denaturing gel electrophoresis. Extension of the labeled probe occurred in a specific manner, limited by the complementarity of the hybridizing sequence.

L36 ANSWER 5 OF 14 CA COPYRIGHT 1996 ACS DUPLICATE 5
AN 119:242202 CA
TI Incorporation of dITP or **7-deaza** dGTP during PCR improves sequencing of the product
AU Dierick, Herman; Stul, Michel; De Kelver, Wim; Marynen, Peter; Cassiman, Jean Jacques
CS Cent. Hum. Genet., Louvain, B-3000, Belg.
SO Nucleic Acids Res. (1993), 21(18), 4427-8
CODEN: NARHAD; ISSN: 0305-1048
DT Journal
LA English
AB Premature enzyme pausing due to regions of complex secondary structure interferes with sequencing reactions. **7-Deaza** dGTP or the more economical dITP analogs were used to generate PCR fragments contg. one of these analogs instead of, or together with , dGTP. The isolated single strand template mols. showed less (than conventional PCR) or no secondary structures, allowing T7 polymerase to read through the entire sequence. Specific expt. conditions required to prevent dITP incorporation, which decreases PCR efficiency and increases misincorporation by **Taq polymerase**, are given. The method was applied to the sequencing of short fragments contg. exon 7 of the p53 gene and of the D-loop region of mitochondrial DNA.

L36 ANSWER 6 OF 14 CA COPYRIGHT 1996 ACS DUPLICATE 6
AN 120:155055 CA
TI Cycle sequencing protocol with fluorescein-12-dCTP
AU Zimmermann, J.; Voss, H.; Wiemann, S.; Erfle, H.; Rupp, T.; Hewitt, N.A.; Schwager, C.; Stegemann, J.; Ansorge, W.
CS Europ. Mol. Biol. Lab., Heidelberg, D-6900, Germany

SO Methods Mol. Cell. Biol. (1993), 4(1), 27-28
CODEN: MMCBEV; ISSN: 0898-7750
DT Journal
LA English
AB A cycle sequencing protocol is reported which uses fluorescein-12-dCTP for direct sequencing of M13, plasmid, and cosmid DNA. This protocol results in better signal intensities and resoln. than do those employing fluorescein-12-**dUTP**. Fluorescein-15-dATP is not well accepted by **Taq polymerase** under conditions of this protocol. Automated base calling can be performed by com. available software.

L36 ANSWER 7 OF 14 CA COPYRIGHT 1996 ACS DUPLICATE 7
AN 119:155382 CA
TI Site-specific incorporation of [125I]iododeoxyuridine into DNA
AU Scherberg, Neal; Bloch, Isac; Gardner, Paul
CS Univ. Chicago Hosp. Clin., Chicago, IL, 60637, USA
SO Appl. Radiat. Isot. (1992), 43(7), 923-7
CODEN: ARISEF; ISSN: 0883-2889
DT Journal
LA English
AB A procedure for the incorporation of [125I]IdU into specific sites in DNA is described. The approach depends upon attachment of radioiododeoxyuridine to a controlled pore glass support which is then used for automated synthesis of an oligomer. The resulting oligomer, contg. a terminal 3'[125I]iododeoxyuridine, is used as a primer during DNA synthesis catalyzed by the **Taq polymerase** employing thermal cycling. The product formed includes the radioiodonucleotide at a single internal site detd. by the length of the oligomer.

L36 ANSWER 8 OF 14 CA COPYRIGHT 1996 ACS DUPLICATE 8
AN 116:208756 CA
TI **7-Deazapurine** containing DNA: efficiency of c7GdTP, c7AdTP and c7IdTP incorporation during PCR-amplification and protection from endodeoxyribonuclease hydrolysis
AU Seela, Frank; Roeling, Angelika
CS Lab. Org. Bioorg. Chem., Univ. Osnabrueck, Osnabrueck, D-4500, Germany
SO Nucleic Acids Res. (1992), 20(1), 55-61
CODEN: NARHAD; ISSN: 0305-1048
DT Journal
LA English
AB The enzymic synthesis of **7-deazapurine** nucleoside-contg. DNA (501 bp) is performed by PCR-amplification (**Taq polymerase**) using a pUC18 plasmid DNA as template and the triphosphates of **7-deaza** -2'-deoxyguanosine (c7Gd), -adenosine (c7Ad) and -inosine (c7Id). C7GdTP can fully replace dGTP resulting in a completely modified DNA-fragment of defined size and sequence. The other two **7-deazapurine** triphosphates (c7AdTP) and (c7IdTP) require the presence of the parent purine 2'-deoxyribonucleotides. In purine/**7-deazapurine** nucleotide mixts.

Taq polymerase prefers purine over 7-**deazapurine** nucleotides but accepts c7GdTP much better than c7AdTP or c7IdTP. As incorporation of 7-**deazapurine** nucleotides represents a modification of the major groove of DNA it can be used to probe DNA/protein interaction. Regioselective phosphodiester hydrolysis of the modified DNA fragments was studied with 28 endodeoxyribonucleases. C7Gd is able to protect the DNA from the phosphodiester hydrolysis in more than 20 cases, only a few enzymes (Mae III, Rsa I, Hind III, Pvu II or Taq I) still hydrolyze the modified DNA. C7Ad protects DNA less efficiently, as this DNA could only be modified in part. The absence of N-7 as potential binding position or a geometric distortion of the recognition duplex caused by the 7-**deazapurine** base can account for protection of hydrolysis.

L36 ANSWER 9 OF 14 CA COPYRIGHT 1996 ACS DUPLICATE 9
AN 116:100248 CA
TI Sequencing self-ligated PCR products using 3' over-hangs generated by specific cleavage of **dUTP** by uracil-DNA glycosylase
AU Day, P. J. R.; Walker, M. R.
CS Dep. Oncol., Child. Hosp., Birmingham, B16 8ET, UK
SO Nucleic Acids Res. (1991), 19(24), 6959
CODEN: NARHAD; ISSN: 0305-1048
DT Journal
LA English
AB PCR products were self-ligated using homopolymer tails generated by the inclusion of 5' polyadenosine and polythymidine sequences followed by deoxyuracil (**dUTP**) to the forward and reverse primer sequences resp. **DUTP** was specifically excised from the PCR primers by using uracil-DNA glycosylase to leave complementary sequences to the homopolymer sequences single-stranded. The homopolymer sequences were chosen to be complementary, and were used to facilitate efficient ligation and subsequent sequencing of 120 bp and 710 bp PCR products. Control reactions omitting ligase did not produce a usable sequencing template. Therefore, because concatemerization was not obsd., the conformation of PCR product formed in the presence of DNA ligase must reduce the rate of PCR product reannealing, thus assisting annealing of the sequencing primer and enabling direct sequencing from either PCR strand. The introduction of an A-A mismatch does not impose a problem for ligation, presumably due to the clamping produced by the polymonobasic sequence. Also, the poor fidelity with which polymerases read **dUTP** may actually increase the likelihood of correctly matched sequences, although this effect has not been obsd. By introducing one or more over-hanging bases into PCR products (as depicted by primer sequence 5' to the uracil moiety), all amplified products will have the appropriate over-hang, rather than relying on the chance probability of nontemplate directed 3' incorporation of adenosine by **Taq polymerase**.
IT **1173-82-6**
RL: USES (Uses)
(of polymerase chain reaction products, uracil-DNA glycosylase

specific cleavage of, DNA sequence detn. in relation to)

L36 ANSWER 10 OF 14 CA COPYRIGHT 1996 ACS DUPLICATE 10
AN 115:200321 CA
TI Improved telomere detection using a telomere repeat probe (TTAGGG)n generated by PCR
AU IJdo, J. W.; Wells, R. A.; Baldini, A.; Reeders, S. T.
CS Howard Hughes Med. Inst., New Haven, CT, 06510, USA
SO Nucleic Acids Res. (1991), 19(17), 4780
CODEN: NARHAD; ISSN: 0305-1048
DT Journal
LA English
AB The rapid generation of human telomere repeat sequence (TTAGGG)n, with fragment sizes up to 25 kb using the polymerase chain reaction (PCR) is reported. This probe can be labeled with biotin-11-**dUTP** or a mixt. of modified eoxynucleotides in the same PCR reaction. Fluorescence in situ hybridization shows signal at all telomers with a signal intensity significantly stronger than that seen using an oligonucleotide probe of the same sequence. The (TTAGGG)n probe is also a useful tool as an anchor point in fluorescence in situ expts. in which several probes are used simultaneously. It allows merging of the different images obtained with several probes labeled with different fluorochromes at the same time. PCR is carried out in the absence of template using primers (TTAGGG)5 and (CCCTAA)5. Staggered annealing of the primers provides a single strand template for extension by **Taq polymerase**. The primers serve as template in the early PCR cycles whereas the newly formed templates serve as primer and template in subsequent stages of the PCR, resulting in a heterogeneous population of mols. consisting of repeat arrays of various lengths. Clearly, reducing the initial primer concn. increases the av. length of the products.

L36 ANSWER 11 OF 14 CA COPYRIGHT 1996 ACS DUPLICATE 11
AN 115:130926 CA
TI Producing single-stranded DNA probes with the Taq DNA polymerase: a high yield protocol
AU Finckh, Ulrich; Lingenfelter, Patricia A.; Myerson, David
CS Fred Hutchinson Cancer Res. Cent., Seattle, WA, 98104, USA
SO BioTechniques (1991), 10(1), 35-6, 38
CODEN: BTNQDO; ISSN: 0736-6205
DT Journal
LA English
AB An efficient procedure is reported to synthesize either single- or double-stranded probes labeled with biotin-11dUTP, biotin-21-**dUTP** or digoxigenin-11dUTP. To produce the single-stranded probe, only a single primer is utilized in a **Taq polymerase** amplification of 55 cycles. A cytomegalovirus probe is presented. This procedure allows easy prodn. of nonradioactivity labeled pure single-stranded probes of any desired length and specificity.

L36 ANSWER 12 OF 14 CA COPYRIGHT 1996 ACS DUPLICATE 12

AN 114:18981 CA
TI Structure-independent DNA amplification by the polymerase chain reaction using **7-deaza** quinine
IN Innis, Michael A.
PA Cetus Corp., USA
SO PCT Int. Appl., 16 pp.
CODEN: PIXXD2
PI WO 9003443 A1 900405
DS W: AU, JP
RW: AT, BE, CH, DE, FR, GB, IT, LU, NL, SE
AI WO 89-US4100 890919
PRAI US 88-248556 880923
DT Patent
LA English
AB DNA sequences that form stable secondary structures, e.g. hairpin loops, and so are not efficiently amplified in std. polymerase chain reaction incubations are efficiently amplified when dGTP is partly replaced by **7-deazadeoxyguanine** triphosphate.
Optimization expts. using the murine ornithine decarboxylase gene showed that a partial substitution of dGTP gave the most efficient results. The amplification of a cDNA for human .beta.-actin was also demonstrated.

L36 ANSWER 13 OF 14 CA COPYRIGHT 1996 ACS DUPLICATE 13
AN 114:18884 CA
TI Preparation of digoxigenin-labeled probes by the polymerase chain reaction
AU Lanzillo, Joseph J.
CS Pulm. Div., New England Med. Cent., Boston, MA, 02111, USA
SO BioTechniques (1990), 8(6), 620, 622
CODEN: BTNQDO; ISSN: 0736-6205
DT Journal
LA English
AB Because of the health hazard, cost and instability assocd. with radio-labeled probes, it is anticipated that they will be replaced ultimately with nonradioactive probes. It is reported here that digoxigenin can be introduced into probes efficiently with digoxigenin-11-**dUTP** (Dg-11-**dUTP**, Boehringer Mannhein, Indianapolis, IN) by the polymerase chain reaction (PCR). Digoxigenin was readily incorporated into the 480 bp-amplified fragment by **Taq polymerase**. Dot blot hybridization with the 50% Dg-labeled probe readily detected 1 pg target DNA by direct chemiluminescence after a 1-h exposure of the blot to x-ray film.

L36 ANSWER 14 OF 14 CA COPYRIGHT 1996 ACS DUPLICATE 14
AN 112:193185 CA
TI Sequencing with Taq DNA polymerase
AU Brow, Mary Ann D.
CS McArdle Lab. Cancer Res., Univ. Wisconsin, Madison, WI, 53706, USA
SO PCR Protoc.: Guide Methods Appl. (1990), 189-96. Editor(s): Innis, Michael A. Publisher: Academic, San Diego, Calif.
CODEN: 56TMAY

DT Conference
LA English
AB Taq DNA polymerase has proven to be highly advantageous for the dideoxynucleotide chain-termination method of DNA sequencing of both conventional and single-stranded PCR templates. The basic sequencing protocol described here involves (1) annealing an oligonucleotide primer to a single-stranded template; (2) labeling the primer in a short, low-temp. polymn. reaction in the presence of .alpha.-labeled dNTP and 3 unlabeled dNTPs, all at low concn.; and (3) extending the labeled primer in 4 sep. base-specific, high-temp. reactions, each in the presence of higher concns. of all dNTPs and 1 chain-terminating ddNTP. If 5'-end-labeled primers are used, step (2) is eliminated. The helix-destabilizing base analog **7-deaza**-2'-deoxyguanosine-5'-triphosphate (c7dGTP) can be incorporated to prevent gel compressions. The products of these reactions are then sepd. by high-resoln. polyacrylamide-urea gel electrophoresis and visualized by autoradiog. or by nonisotopic detection methods.

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L40 3 FILE LIFESCI

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L47  ANSWER 1 OF 19  BIOSIS  COPYRIGHT 1996 BIOSIS     DUPLICATE 1
AN   94:161547  BIOSIS
DN   97174547
TI   A more reliable PCR for detection of Mycobacterium tuberculosis in
     clinical samples.
AU   Kox L F F; Rhienthong D; Miranda A M; Udomasantisuk N; Ellis K; Van
     Leeuwen J; Van Heusden S; Kuijper S; Kolk A H J
CS   Royal Tropical Inst., N.H. Swellengrebel Lab. Tropical Hygiene,
     Meibergdref 39, 1105 AZ Amsterdam, NET
SO   Journal of Clinical Microbiology 32 (3). 1994.  672-678. ISSN:
     0095-1137
LA   English
AB   Diagnostic techniques based on PCR have two major problems:
     false-positive reactions due to contamination with DNA fragments from
     previous PCRs (amplicons) and false-negative reactions caused by
     inhibitors that interfere with the PCR. We have improved our
     previously reported PCR based on the amplification of a fragment of
     the Mycobacterium tuberculosis complex-specific insertion element
     IS6110 with respect to both problems. False-positive reactions caused
     by amplicon contamination were prevented by the use of
     uracil-N-glycosylase and dUTP instead of dTTP. We selected
     a new set of primers outside the region spanned by the formerly used
     primers to avoid false-positive reactions caused by dTTP-containing
     amplicons still present in the laboratory. With this new primer set,
     16 copies of the IS6110 insertion element, the equivalent of two
     bacteria, could be amplified 10-10 times in 40 cycles, resulting in a
     mean efficiency of 77% per cycle. To detect the presence of
     inhibitors of the Taq polymerase, which may cause
     false-negative reactions, part of each sample was spiked with M.
     tuberculosis DNA. The DNA purification method using guanidinium
     thiocyanate and diatoms effectively removed most or all inhibitors of
     the PCR. However, this was not suitable for blood samples, for which
     we developed a proteinase K treatment followed by phenol-chloroform
     extraction. This method permitted detection of 20 M. tuberculosis
```

bacteria per ml of whole blood. Various laboratory procedures were introduced to reduce failure or inhibition of PCR and avoid DNA cross contamination. We have tested 218 different clinical specimens obtained from patients suspected of having tuberculosis. The samples included sputum (n = 145), tissue biopsy samples (n = 25), cerebrospinal fluid (n = 15), blood (n = 14), pleural fluid (n = 9), feces (n = 7), fluid from fistulae (n = 2), and pus from a wound (n = 1). The results obtained by PCR were consistent with those obtained with culture, which is the "gold standard." We demonstrate that PCR is a useful technique for the rapid diagnosis of tuberculosis at various sites.

L47 ANSWER 2 OF 19 MEDLINE
AN 94325338 MEDLINE
TI Comparison of the sequence specificity of cis-diamminedichloroplatinum (II) damage in guanine- and **7-deazaguanine**-containing DNA.
AU Cairns M J; Murray V
CS School of Biochemistry and Molecular Genetics, University of New South Wales, Kensington, Australia..
SO BIOCHIMICA ET BIOPHYSICA ACTA, (1994 Aug 2) 1218 (3) 315-21. Journal code: A0W. ISSN: 0006-3002.
CY Netherlands
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 9411
AB The N7 of guanine is thought to be the primary target for adduct and crosslink formation between cisplatin and DNA. However, reactive sites in DNA other than the N7 of guanine may also participate in the formation of adducts with cisplatin. The possibility that these interactions arise and form DNA polymerase blocking lesions was investigated by primer extension reactions with Taq DNA polymerase. To differentiate between damage produced at relatively weak sites from those formed at the N7 of guanine, a modified DNA template was synthesised with the N7 of guanine replaced with a carbon atom. This was achieved in a PCR designed to incorporate **7-deazaguanine** instead of normal guanine. The sequence specificity of cisplatin damage in the modified and unmodified DNA substrates was compared (after linear amplification) by DNA sequencing gel analysis. For concentrations of cisplatin (1 to 5 microM) that induce blocking lesions in normal DNA, no significant damage was observed in the modified DNA. This confirmed that the N7 of guanine is the major site of adduct formation in normal DNA. At higher concentrations of cisplatin (50 microM and 100 microM), lesions were found at AA dinucleotides and other novel sites in the modified DNA. These results indicate that the N7 of guanine is not required in the formation of some cisplatin adducts.

L47 ANSWER 3 OF 19 BIOSIS COPYRIGHT 1996 BIOSIS DUPLICATE 2
AN 95:58783 BIOSIS
DN 98073083
TI In situ transcription with Tth DNA polymerase and fluorescent

nucleotides.
AU Chang H
CS Naval Med. Res. Inst., Code 63, Bethesda, MD 20889-5607, USA
SO Journal of Immunological Methods 176 (2). 1994. 235-243. ISSN: 0022-1759
LA English
AB We and others have described methods to label specific nucleic acid sequences in fixed cells by reverse in situ transcription (IST). They are simple alternatives to the tedious steps of in situ hybridization with labeled probes. We have favored use of thermostable DNA polymerases after heat denaturation of template secondary structure, accompanied by synthesis of cDNA from an annealed primer, but the approach has been limited by the low reverse transcriptase (RT) activity of **Taq polymerase** and delayed detection methods. We have improved the technique by the use of recombinant Thermus themophilus (rTth) DNA polymerase and fluorescein-12-**dUTP** (FIST). Jurkat T lymphocytes were stimulated with ionomycin + phorbol myristate acetate to produce interleukin-2 (IL-2) mRNA in vitro overnight. They were cytospun onto slides and fixed in 70% ethanol + 30% DEPC-treated water, acetone, and air-dried. The slides were placed on a temperature-controlled heating block, and the cell spot was covered with a plastic coverslip. The temperature was raised to 95 degree C, and 5-10 mu-l of modified Perkin-Elmer/Cetus rTth RT reaction mix was injected under the edge of the coverslip. Each 10 MU-l of mix in DEPC-water contained 10 mM Tris-HCl, pH 8.3, 90 mM KCl, 1 mM MnCl-2, 1 mM dithiothreitol, 10 U placental ribonuclease inhibitor, 0.125 mM dA,C,GTPs, 0.1 mM fluorescein-12-**dUTP**, 2 U rTth DNA polymerase, and 4 pM 22-mer oligonucleotide primer, which spanned the second intron of IL-2. After 3 min at 95 degree C, 1 min at 50 degree C and 10 min at 72 degree C, the slides were washed in 0.5 times phosphate-buffered saline, pH 7.0, at 42 degree C, in 70% ethanol, 100% ethanol, and air-dried. The cells were mounted in antifade solution (2% n-propyl gallate in 70% glycerol), and could be viewed immediately by fluorescence microscopy. Image analysis showed that stimulated Jurkat cells were brighter than uninduced controls or those treated with RNase or without polymerase or primer. FIST appears to be useful for the detection of specific mRNAs in single cells.

L47 ANSWER 4 OF 19 MEDLINE
AN 94367331 MEDLINE
TI Use of a nonradioactive genetic probe identified, synthesized, and labeled in the polymerase chain reaction.
AU Preus H R; Russell D T
CS Department of Periodontology, Dental Faculty, University of Oslo, Norway..
SO SCANDINAVIAN JOURNAL OF DENTAL RESEARCH, (1994 Jun) 102 (3) 161-7. Journal code: UCQ. ISSN: 0029-845X.
CY Denmark
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Dental Journals
EM 9412

AB This study introduces a strategy to identify and produce sequences useful as genetic markers, or native genetic probes for DNA-DNA hybridization in bacterial strains where the genetics is not well described. Actinobacillus actinomy-cetemcomitans (A.a.) was used as an example. Fifty ng genomic DNA from A.a. ATCC 33384 and Haemophilus aphrophilus ATCC 33389 was amplified in a thermocycler using a single 10-mer primer. The PCR products were separated by electrophoresis on a 1% submarine agarose gel containing ethidium bromide and visualized by UV illumination, and the strain-specific amplitypes were compared. DNA from two bands, 0.9 and 4 kb, unique for the A.a. strain, was cut out, amplified under high stringency with the same primer and labeled by replacing 33.3 microM dTTP with digoxigenin-labeled **dUTP** in the reaction mixture. The labeled probe was then repeatedly used for hybridization to DNA from various A.a., H. aphrophilus, and other bacterial strains of the Pasteurellaceae family. The results showed that the 0.9-kb probe detected all A.a. tested, and distinguished it from other closely related bacterial species. We conclude that the described strategy is useful for identifying and selecting genetic sequences useful as genetic markers in A.a.

L47 ANSWER 5 OF 19 EMBASE COPYRIGHT 1996 ELSEVIER SCI. B.V.
AN 94216013 EMBASE
TI [Intracellular PCR: A new approach for diagnoses].
PCR INTRACELLULAIRE: NOUVELLE APPROCHE DE DIAGNOSTIC TISSULAIRE ET CYTOGENETIQUE.
AU Teyssier M.; Carosella E.; Gluckman E.; Kirszenbaum M.
CS CEA, Laboratoire d'Immunoradiobiologie, Hopital Saint-Louis, 1, Avenue Claude-Vellefaux, 75475 Paris Cedex 10, France
SO IMMUNO-ANAL. BIOL. SPEC., (1994) 9/3 (159-164).
ISSN: 0923-2532 CODEN: IBSPEW
CY France
DT Journal
FS 022 Human Genetics
026 Immunology, Serology and Transplantation
LA French
SL French; English
AB The aim of this study was to improve the technique of in situ polymerase chain reaction (PCR) on glass slides in order to detect CD34 and c-kit genes on individual mononuclear cells (MNC). This method appears to increase the sensitivity of ISH without loss of morphology. MNC were deposited on slides, fixed and permeabilized. After in situ PCR, hybridization was realized with a digoxigenin-**dUTP**(DIG)-labelled probe. Detection was mediated by an anti-DIG antibody conjugate to alkaline phosphatase and the color substrate of this enzyme. It is worthy to mention that some PCR products diffused out of the cells in which amplification occurs and could be detected by electrophoresis of the supernatant. This direct qualitative control approach led to rapid visualization of amplified products before subsequent ISH. The PCR positive cells were identified by a black-blue coloration, whereas with the same hybridization, cells that undergone PCR without **Taq polymerase** remained uncolored.

L47 ANSWER 6 OF 19 BIOSIS COPYRIGHT 1996 BIOSIS DUPLICATE 3
AN 94:440030 BIOSIS
DN 97453030
TI Polymerase chain reaction-based diagnostic assay to detect cattle chronically infected with Babesia bovis.
AU Figueroa J V; Chieves L P; Johnson G S; Goff W L; Buening G M
CS CENID-PAVET, INIFAP-SARH. Apdo. Postal No. 206 CIVAC, Morelos, 62500, MEX
SO Revista Latinoamericana de Microbiologia 36 (1). 1994. 47-55. ISSN: 0034-9771
LA English
AB From a B. bovis gene sequence coding for a 60 kDa merozoite surface protein previously published, two sets of primers were designed for the Polymerase Chain Reaction (PCR) assay. Primer set BoF/BoR was used to prime **Taq Polymerase** DNA amplification of a 350 bp fragment of the target B. bovis DNA. Primer set BoFN/BoRN was used to prepare a PCR-sinthesized, Digoxigenin-**dUTP** -labeled probe (291 bp) which would hybridize to a sequence within the PCR-amplified parasite target DNA. PCR amplification of target DNA obtained from in vitro-cultured B. bovis and nucleic acid hybridization of amplified product with the nonradioactive DNA probe showed that a 350 bp fragment could be detected when as little as 10 pg of genomic parasite DNA was utilized in the assay. A fragment of similar size was amplified from genomic DNA from four other B. bovis isolates but not from B. bigemina, Anaplasma marginale, or bovine leukocyte DNA. The PCR product was detected in blood samples containing approximately 3 B. bovis-infected erythrocytes (20 mu-l of packed cells with a parasitemia of 0.000001%). By using the PCR/DNA probe assay, 16 out of 20 animals experimentally inoculated with B. bovis were detected positive, whereas no PCR product was observed in bovine blood sample collected from 20 B. bigemina-infected, and 20 uninfected cattle tested. The PCR-DNA probe assay was shown to be sensitive in detecting some cattle with B. bovis-chronic infection. The specificity and high analytical sensitivity of the test provides a valuable tool to apply in conducting epidemiological studies.

L47 ANSWER 7 OF 19 MEDLINE
AN 93324337 MEDLINE
TI Elimination of band compression in sequencing gels by the use of N4-methyl-2'-deoxycytidine 5'-triphosphate.
AU Li S; Haces A; Stupar L; Gebeyehu G; Pless R C
CS Life Technologies, Inc., Gaithersburg, MD 20884-9980..
SO NUCLEIC ACIDS RESEARCH, (1993 Jun 11) 21 (11) 2709-14. Journal code: O8L. ISSN: 0305-1048.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 9310
AB Taq DNA polymerase, Sequenase, and the large fragment of E.coli polymerase I effectively utilize N4-methyl-2'-deoxycytidine 5'-triphosphate (N4-methyl-dCTP) in the place of dCTP in

dideoxynucleotide terminator sequencing reactions on single-stranded templates. When the resulting fragment mixtures are resolved on sequencing gels, they are found to be free of band compressions even in cases where such compressions remain unresolved by the substitution of **7-deaza**-dGTP for dGTP. Sequencing reactions using N4-methyl-dCTP instead of dCTP are somewhat more prone to false stops than are sequencing reactions using **7-deaza**-dGTP instead of dGTP; this difference is more pronounced when sequencing with Sequenase at 37 degrees C than when sequencing with Taq DNA polymerase at 72 degrees C. For the three polymerases investigated, replacement of dCTP by N4-methyl-dCTP does not fundamentally change the characteristic variations in band intensities seen in the C-lane. N4-methyl-dCTP can also be used for sequencing double-stranded DNA and for DNA amplification by the polymerase chain reaction.

L47 ANSWER 8 OF 19 MEDLINE
AN 93356464 MEDLINE
TI Pitfalls of in situ polymerase chain reaction (PCR) using direct incorporation of labelled nucleotides.
AU Sallstrom J F; Zehbe I; Alemi M; Wilander E
CS Department of Pathology, University Hospital, Uppsala, Sweden..
SO ANTICANCER RESEARCH, (1993 Jul-Aug) 13 (4) 1153.
Journal code: 59L. ISSN: 0250-7005.
CY Greece
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 9311
AB False positivity is reported of in situ PCR reactions in a direct incorporation assay with digoxigenin-labelled **dUTP**. It is recommended that in situ hybridization with specific labelled probe replaces the direct incorporation method for the detection of in situ PCR amplicon.

L47 ANSWER 9 OF 19 MEDLINE
AN 93207760 MEDLINE
TI Overcoming GC compression in nucleotide sequencing.
AU Beck K F; Stathopulos I; Berninger M G; Schweizer M
CS Institut fur Mikrobiologie und Biochemie, Universitat Erlangen-Nurnberg, FRG..
SO BIOTECHNIQUES, (1993 Mar) 14 (3) 375.
Journal code: AN3. ISSN: 0736-6205.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 9307

L47 ANSWER 10 OF 19 LIFESCI COPYRIGHT 1996 CSA
AN 93:132239 LIFESCI
TI Cycle sequencing protocol with fluorescein-12-dCTP.
AU Zimmermann, J.; Voss, H.; Wiemann, S.; Erfle, H.; Rupp, T.; Hewitt,

N.A.; Schwager, C.; Stegemann, J.; Ansorge, W.
CS Eur. Mol. Biol. Lab., D-6900 Heidelberg, FRG
SO METHODS MOL. CELL. BIOL., (1993) vol. 4, no. 1, pp. 27-28. ISSN: 0898-7750.
DT Journal
FS N; G; G3
LA English
AB Recently we have introduced procedures for automated DNA sequencing with internal labeling and T7 DNA polymerase, using fluorescein-12-**dUTP** and fluorescein-15-dATP without the need of labeled primers or fluorescent dye terminators. Cycle sequencing protocols were developed for automated DNA sequencing, allowing the sequencing of small amounts of DNA. The principle of internal labeling for cycle sequencing with Taq DNA polymerase was shown with fluorescein-12-**dUTP**. Here we report a cycle sequencing protocol using the fluorescein-12-dCTP, which we applied to direct sequencing of M13, plasmid, and cosmid DNA. This protocol results in better signal intensities and resolution than those employing fluorescein-12-**dUTP**. Fluorescein-15-dATP is not well accepted by **Taq polymerase** under conditions of this protocol. Automated base calling can be performed by commercially available software (A.L.F., Pharmacia).

L47 ANSWER 11 OF 19 WPIDS COPYRIGHT 1996 DERWENT INFORMATION LTD
AN 92-064970 [08] WPIDS
CR 88-058187 [09]; 89-233845 [32]; 91-222898 [30]; 91-222902 [30]; 92-096899 [12]; 92-150885 [18]; 92-150887 [18]; 94-150436 [18]; 95-154582 [20]
DNC C92-029827
TI Reducing non-specific nucleic acid amplification - by inserting modified nucleic acid bases into reaction mixt and treating prods. to prevent their action as templates.
DC B04 D16
IN GELFAND, D H; KWOK, S Y; SNINSKY, J J
PA (CETU) CETUS CORP; (HOFF) HOFFMANN LA ROCHE & CO AG F; (HOFF) HOFFMANN LA ROCHE INC
CYC 18
PI WO 9201814 A 920206 (9208)*
RW: AT BE CH DE DK ES FR GB GR IT LU NL SE
W: AU CA JP US
AU 9185327 A 920218 (9222)
EP 540693 A1 930512 (9319) EN 56 pp
R: AT BE CH DE DK ES FR GB GR IT LI LU NL SE
JP 06501612 W 940224 (9413) 28 pp
WO 9201814 A3 920514 (9510)
US 5418149 A 950523 (9526) 23 pp
AU 665338 B 960104 (9608)
ADT AU 9185327 A AU 91-85327 910723, WO 91-US5210 910723; EP 540693 A1 EP 91-916353 910723, WO 91-US5210 910723; JP 06501612 W JP 91-515604 910723, WO 91-US5210 910723; WO 9201814 A3 WO 91-US5210 910723; US 5418149 A CIP of US 90-557517 900724, CIP of US 90-609157 901102, WO 91-US5210 910723, US 93-960362 930105; AU 665338 B AU 91-85327 910723

FDT AU 9185327 A Based on WO 9201814; EP 540693 A1 Based on WO 9201814; JP 06501612 W Based on WO 9201814; US 5418149 A Based on WO 9201814; AU 665338 B Previous Publ. AU 9185327, Based on WO 9201814

PRAI US 90-609157 901102; US 90-557517 900724; US 93-960362 930105

AN 92-064970 [08] WPIDS

CR 88-058187 [09]; 89-233845 [32]; 91-222898 [30]; 91-222902 [30]; 92-096899 [12]; 92-150885 [18]; 92-150887 [18]; 94-150436 [18]; 95-154582 [20]

AB WO 9201814 A UPAB: 931006

Reducing non-specific amplification in a primer-based amplification reaction comprises; (a) incorporating a modified nucleoside triphosphate (nTP) and a glycosylase specific for the nTP, in the reaction; (b) incubating the reaction of step (a) at a temp. below that of denaturation of the glycosylase and below that of specific hybridisation of the primers to render the modified nucleotides in the amplification prods. basic; (c) inactivating the glycosylase; and (d) incubating the reaction of step (c) at the temp. for specific hybridisation of the primers.

Also claimed are; (1) sterilising a nucleic acid (NA) amplification reaction system, comprising degrading the contaminating amplified prod. by hydrolysing covalent bonds of the unconventional nucleotides; (2) improved amplification methods, generating amplified NA that can be rendered unamplifiable, preventing deleterious effects from the amplified NA contaminating subsequent amplifications; (3) purifying a recombinant protein (A) from a host cell.

ADVANTAGE - Improved amplification methods can reduce non-specific amplification of NAs as well as minimising the effects of contamination with previously generated prods.. Methods allow an enhanced specificity of NA amplification assays. Na-free proteins can be produced which are useful as reagents for amplification systems.

0/0

ABEQ EP 540693 A UPAB: 931113

Reducing non-specific amplification in a primer based amplification reaction comprises: (a) incorporating a modified nucleoside triphosphate (nTp) and a glycosylase specific for the nTP, in the reaction; (b) incubating the reaction of step (a) at a temp. below that of denaturation of the glyosylase and below that of specific hybridisation of the primers to render the modified nucleotides in the amplification prods, basic; (c) inactivating the glycosylase; and (d) incubating the reaction of step (c) at temp. for specific hybridisation of the primers.

Also claimed are: (1) sterilising a nucleic acid (NA) amplification reaction system, comprising degrading the contaminating amplified prod. by hydrolysing covalent bonds of the unconventional nucleotides; (2) improved amplification methods, generating amplified NA that can be rendered unamplifiable, preventing deleterious effects from the amplified NA contaminating subsequent amplifications; and (3) purifying a recombinant protein (A) from a host cell.

ADVANTAGE - Improved amplification methods can reduce non-specific amplification of NAs as well as minimising the effects

of contamination with previously generated prods. Methods allow an enhanced specificity of NA amplification assays. Na-free proteins can be produced which are useful as reagents for amplification.

ABEQ US 5418149 A UPAB: 950705

Redn. of non-specific amplification in primer-based amplification comprises incubation of the amplification mixt. in the presence of a modified nucleoside triphosphate (**dUTP**) so that the synthetic nucleic acid contains a modified nucleotide and also a glycosylase (UNG) that degrades nucleic acid contg. the modified nucleotide at 45-60 deg.C until all nucleic acid contg. the modified nucleotide has been degraded, then further amplification at temps. above 55 deg.C. Pref. reaction are chain reactions involving a thermally stable DNA polymerase.

USE/ADVANTAGE - The process is applicable to nucleic acid synthesis by means of chain reactions with **Taq polymerase**. The process avoids the accumulation of non-specific templates, giving synthetic nucleic acids of improved purity.

Dwg.0/0

L47 ANSWER 12 OF 19 BIOTECHDS COPYRIGHT 1996 DERWENT INFORMATION LTD

AN 92-13390 BIOTECHDS

TI Improved efficiency in amplification of ancient DNA and its sequence analysis;
using the polymerase chain reaction

AU Hummel S; Nordsiek G; Herrmann B

LO Institut fuer Anthropologie der Universitaet, W-3400 Goettingen, Germany.

SO Naturwissenschaften; (1992) 79, 8, 359-60
CODEN: NATWAY

DT Journal

LA English

AN 92-13390 BIOTECHDS

AB A modified 'booster' polymerase chain reaction (PCR) protocol was suggested for amplifying DNA from ancient sources. A first series of 10 cycles was performed with a regular primer concentration, regular dNTP content and regular, buffered reaction mix, with 1% of the DNA deriving from about 0.25 g of 4-day EDTA-extracted bone powder and 1.0 U of Taq DNA-polymerase (EC-2.7.7.7). The stringency of the reaction was low in these first 10 cycles. A second series of 20-25 cycles was performed with high stringency parameters, lowered primer concentrations and 1.5-2.0 U **Taq polymerase**, with 10% of the initial 50 ul reaction used as template. DNA extracted from ancient bones of females and males was processed. Primers specific for the Y-chromosome, yielded amplification products from all male but no female bone extracts. For cloning, these amplified products were treated with phage T7 DNA-polymerase, blunt-end ligated into vector phasmid pmC5-delta bla ac and transformed into Escherichia coli SURE. Automated sequencing was performed using T7 polymerase with internal labeling of single-stranded DNA using fluorescein-12-**dUTP**. (16 ref)

L47 ANSWER 13 OF 19 BIOSIS COPYRIGHT 1996 BIOSIS DUPLICATE 4
AN 92:184752 BIOSIS
DN BA93:95702
TI **7 DEAZAPURINE** CONTAINING DNA EFFICIENCY OF C-7G-DTP C-7A-DTP AND C-7I-DTP INCORPORATION DURING PCR-AMPLIFICATION AND PROTECTION FROM ENDODEOXYRIBONUCLEASE HYDROLYSIS.
AU SEELA F; ROELING A
CS LAB. ORGANISCHE BIOORGANISCHE CHEMIE, FACHBEREICH BIOLOGIE/CHEMIE, UNIVERSITAET OSNABRUCK, BARBARASTRASSE 7, D-4500 OSNABRUCK, GER.
SO NUCLEIC ACIDS RES 20 (1). 1992. 55-61. CODEN: NARHAD ISSN: 0305-1048
LA English
AB The enzymatic synthesis of **7-deazapurine** nucleoside containing DNA (501 bp) is performed by PCR-amplification (**Taq polymerase**) using a pUC18 plasmid DNA as template and the triphosphates of **7-deaza** -2'-deoxyguanosine (c7Gd), -adenosine (c7Ad) and -inosine (c7Id). c7GdTP can fully replace dGTP resulting in a completely modified DNA-fragment of defined size and sequence. The other two **7-deazapurine** triphosphates (c7AdTP) and (c7dTP) require the presence of the parent purine 2'-deoxyribonucleotides. In purine/ **7-deazapurine** nucleotide mixtures **Taq polymerase** prefers purine over **7-deazapurine** nucleotides but accepts c7GdTP much better than c7AdTP or c7dTP. As incorporation of **7-deazapurine** nucleotides represents a modification of the major groove of DNA it can be used to probe DNA/protein interaction. Regioselective phosphodiester hydrolysis of the modified DNA-fragments was studied with 28 endodeoxyribonucleases. c7Gd is able to protect the DNA from the phosphodiester hydrolysis in more than 20 cases, only a few enzymes (Mae III, Rsa I, Hind III, Pru II or Taq I) do still hydrolyze the modified DNA. c7Ad protects DNA less efficiently, as this DNA could only be modified in part. The absence of N-7 as potential binding position or a geometric distortion of the recognition duplex caused by the **7-deazapurine** base can account for protection of hydrolysis.

L47 ANSWER 14 OF 19 WPIDS COPYRIGHT 1996 DERWENT INFORMATION LTD
AN 91-095712 [14] WPIDS
DNC C91-040924
TI Nucleic acid hybridisation assays - using a capture probe immobilised on a solid support to bind a labelled target nucleic acid sequence.
DC B04 D16
IN LONGIARU, M; SILVER, S B; SULZINSKI, M A
PA (HOFF) HOFFMANN LA ROCHE & CO AG F; (HOFF) HOFFMANN-LA ROCHE AG; (HOFF) HOFFMANN LA ROCHE INC
CYC 21
PI EP 420260 A 910403 (9114)*
R: AT BE CH DE ES FR GB IT LI NL SE
AU 9063290 A 910411 (9122)
NO 9004240 A 910402 (9122)
CA 2026280 A 910330 (9124)
ZA 9007706 A 910626 (9132)

BR 9004881 A 910910 (9141)
JP 03206898 A 910910 (9142)
US 5232829 A 930803 (9332) 15 pp
NZ 235463 A 940325 (9426)
NZ 247522 A 940325 (9426)
IL 95800 A 941007 (9445)
AU 9472804 A 941124 (9503)

ADT EP 420260 A EP 90-118620 900927; ZA 9007706 A ZA 90-7700 900926; JP 03206898 A JP 90-262934 900929; US 5232829 A US 89-414542 890929; NZ 235463 A NZ 90-235463 900926; NZ 247522 A NZ 90-247522 900926; IL 95800 A IL 90-95800 900926; AU 9472804 A AU 94-72804 940902, Div ex AU 90-63290

FDT NZ 247522 A Div ex NZ 235463

PRAI US 89-414542 890929

AN 91-095712 [14] WPIDS

AB EP 420260 A UPAB: 950110

(A) a method of detecting a labelled target nucleic acid sequence amplified from a biological sample comprising hybridising the target nucleic acid sequence with at least one oligonucleotide capture probe having a nucleic acid sequence complementary to the target sequence, the capture probe being bound to a polystyrene solid support, and determining the presence of the label associated with the target sequence; the hybridisation may be conducted in the presence of guanidine thiocyanate; the label may be biotin and the target sequence may be labelled by biotin-11-**dUTP** incorporation by **Taq polymerase** during polymerase chain reaction (PCR) amplification; the biotin label may be detected by addn. of avidin or streptavidin complexed with horseradish peroxidase (HRP), alkaline phosphatase, beta-galactosidase, luciferase, fluorescein or Texas red; the HRP may be detected with a chromogenic agent, e.g. 3,3',5,5'-tetramethyl benzidine or o-phenylene diamine and a substrate, e.g. H_2O_2.

(B) a method of quantitatively determining the extent of hybridisation of a target nucleic acid sequence in a capture probe assay which comprises (a) utilising microtitre plates as the support for passively bound capture probes, (b) contacting the plates with a biological sample contg. a target nucleic acid sequence which has been labelled to generate a signal capable of detection and (c) counting or otherwise measuring in quantitative fashion the signals so generated.

USE/ADVANTAGE - The method is used for the labelling and detection of target sequences, e.g. Chlamydia trachomatis nucleic acid. The plate assay is highly accurate and sensitive and can be accomplished in 2hrs.. The capture of the labelled target sequences provides an objective, quantitative evaluation of hybridisation, the calculation of a statistical cut-off point for positivity of samples and the quantitative calculation of an assay ''signal-to-noise'' ratio. @(21pp Dwg.No.0/3)

0/3

ABEQ US 5232829 A UPAB: 931118

Amplified DNA is produced by a polymerase chain reaction, labelled (e.g. with biotin), then hybridised with a base pair to an amplicon-specific oligonucleotide that has been immobilised on a

solid polystyrene support, pref. on well walls in a microtitration plate.

USE - The process is applicable to DNA from Chlamydia trachomatis, and the resulting microtitration plates provide kits for the rapid clinical diagnosis of Chlamydia trachomatis infection. Dwg.0/3

L47 ANSWER 15 OF 19 MEDLINE
AN 93027441 MEDLINE
TI Solid-phase synthesis of oligo(2'-deoxyxylonucleotides) and PCR amplification of base-modified DNA fragments.
AU Seela F; Rosemeyer H; Krecmerova M; Roling A
CS Laboratorium fur Organische und Bioorganische Chemie, Fachbereich Biologie/Chemie, Universitat Osnabruck, FRG..
SO NUCLEIC ACIDS SYMPOSIUM SERIES, (1991) (24) 87-90. Journal code: O8N. ISSN: 0261-3166.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 9301
AB 1-(2'-Deoxy-beta-D-threo-pentofuranosyl)thymine (xTd) and -adenine (xAd) were converted into their appropriately protected 3'-phosphonates 1a, 2a as well as their 2-cyanoethyl phosphoramidites 1b, 2b. These compounds were used for solid-phase syntheses of the oligo(2'-deoxy-beta-D-xylonucleotides) 5-8. Structural properties and behavior against nucleases is described. Apart from oligo(2'-deoxyxylonucleotides) the PCR-amplification of a pUC18 DNA fragment with **Taq polymerase** was studied in the presence of the **7-deazapurine** derivatives of dGTP, dATP, and dITP. The incorporation efficiency of the modified compounds was compared with those of the parent nucleotides. **7-Deaza**-2'-deoxyguanosine protected the DNA-fragment from hydrolysis by the restriction endodeoxyribonuclease Eco RI, Pst I, Bam HI, and Sma I if the nucleoside was located within the recognition site.

L47 ANSWER 16 OF 19 BIOSIS COPYRIGHT 1996 BIOSIS DUPLICATE 5
AN 91:318189 BIOSIS
DN BA92:28704
TI PRODUCING SINGLE-STRANDED DNA PROBES WITH THE TAQ DNA POLYMERASE A HIGH YIELD PROTOCOL.
AU FINCKH U; LINGENFELTER P A; MYERSON D
CS FRED HUTCHINSON CANCER RES. CENT., 1124 COLUMBIA ST., SEATTLE, WASHINGTON 98104.
SO BIOTECHNIQUES 10 (1). 1991. 35-36, 38-39. CODEN: BTNQDO ISSN: 0736-6205
LA English
AB We report on efficient procedure to synthesize either single- or double-stranded probes labeled with biotin-11-**dUTP**, biotin-21-**dUTP** or digoxigenin-11-**dUTP**. To produce the single-stranded probe, only a single primer is utilized in a **Taq polymerase** amplification of 55 cycles. A

cytomegalovirus probe is presented. This procedure allows easy production of nonradioactively labeled pure single-stranded probes of any desired length and specificity.

L47 ANSWER 17 OF 19 SCISEARCH COPYRIGHT 1996 ISI (R)
AN 91:52195 SCISEARCH
GA The Genuine Article (R) Number: ET526
TI PRODUCING SINGLE-STRANDED-DNA PROBES WITH THE TAQ DNA-POLYMERASE - A HIGH-YIELD PROTOCOL
AU FINCKH U; LINGENFELTER P A; MYERSON D (Reprint)
CS FRED HUTCHINSON CANC RES CTR, 1124 COLUMBIA ST, SEATTLE, WA, 98104; FREE UNIV BERLIN, KLINIKUM RUDOLF VIRCHOW, DEPT INNERE MED HAMATOL ONKOL, W-1000 BERLIN 33, GERMANY; UNIV WASHINGTON, DEPT PATHOL, SEATTLE, WA, 98195
CYA USA; GERMANY
SO BIOTECHNIQUES, (1991) Vol. 10, No. 1, pp. 35.
DT Note; Journal
FS LIFE
LA ENGLISH
REC Reference Count: 11
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB We report an efficient procedure to synthesize either single- or double-stranded probes labeled with biotin-11-**dUTP**, biotin-21-**dUTP** or digoxigenin-11-**dUTP**. To produce the single-stranded probe, only a single primer is utilized in a **Taq polymerase** amplification of 55 cycles. A cytomegalovirus probe is presented. This procedure allows easy production of nonradioactively labeled pure single-stranded probes of any desired length and specificity.

L47 ANSWER 18 OF 19 MEDLINE DUPLICATE 6
AN 90334370 MEDLINE
TI Detection of Rickettsia tsutsugamushi by gene amplification using polymerase chain reaction techniques.
AU Kelly D J; Marana D P; Stover C K; Oaks E V; Carl M
CS Rickettsial Diseases Division, Naval Medical Research Institute, Bethesda, Maryland 20814-5055..
SO ANNALS OF THE NEW YORK ACADEMY OF SCIENCES, (1990) 590 564-71. Journal code: 5NM. ISSN: 0077-8923.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 9011
AB Scrub typhus is commonly undiagnosed in endemic areas due, in part, to dependence on retrospective serodiagnosis. Since the etiologic agent, R. tsutsugamushi, will not grow in cell-free systems, a rapid direct-agent detection system such as provided by polymerase chain reaction (PCR) methodology is needed. Genes coding for the variable 56-kDa antigen of R. tsutsugamushi were amplified through 35 cycles using 20-mer oligonucleotide primers and **Taq polymerase**. Amplification of 1-ng samples of DNA extracted from purified prototype R. tsutsugamushi Karp, Gilliam, and Kato

strains was detected by direct visual inspection of the electrophoresed, ethidium bromide-stained, specific bands. Specificity of the PCR was shown when PCR amplification of various non-scrub typhus rickettsial DNAs was unsuccessful. R. tsutsugamushi DNA extracted from the blood of infected mice could be PCR amplified and the 1477-base pair product detected by either direct visualization or by specific hybridization with amplified non-radioactive digoxigenin-11-**dUTP**-labeled Karp 56-kDa DNA probe.

L47 ANSWER 19 OF 19 LIFESCI COPYRIGHT 1996 CSA
AN 88:81072 LIFESCI
TI DNA sequencing using **Taq polymerase**.
AU Peterson, M.G.
CS Walter and Eliza Hall Inst. Med. Res., PO Royal Melbourne Hosp., Vic. 3050, Australia
SO NUCLEIC ACIDS RES., (1988) vol. 16, no. 22, p. 10915.
DT Journal
FS N; W
LA English
AB Three DNA polymerases, namely E. coli DNA polymerase 1 (Klenow), reverse transcriptase and T7 DNA polymerase (sequenase), are commonly used for DNA sequencing by the chain termination method of Sanger and colleagues. However, the secondary structure of the DNA template can impede the progress of all three polymerases. The author has developed a novel procedure in which the thermostable polymerase of Thermus aquaticus (**Taq polymerase**) is utilized in a reaction incorporating **7-deaza** dGTP and performed at 70 degree C, at which the effects of secondary structure are eliminated.

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E2	2		BROW K C/AU
E3	0	-->	BROW M/AU
E4	2		BROW M A D/AU
E5	2		BROW MARK J/AU
E6	8		BROW MARY ANN/AU
E7	22		BROW MARY ANN D/AU
E8	2		BROW MARYANN D/AU
E9	2		BROW R E/AU
E10	68		BROW R K/AU

-Author (S)

=> s e4-e8; e lyamicher v/au 5

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L49          18 FILE CAPLUS

TOTAL FOR ALL FILES
L50          36 ("BROW M A D"/AU OR "BROW MARK J"/AU OR "BROW MARY ANN"/AU
                OR "BROW MARY ANN D"/AU OR "BROW MARYANN D"/AU)


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E10           2      LYAMIN A M/AU

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L52          32 FILE CAPLUS

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                OR"/AU OR "LYAMICHEV VICTOR I"/AU)


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E3           76 --> OLIVE D/AU
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E6            2      OLIVE D L/AU
E7            6      OLIVE D M/AU
E8           24      OLIVE D MICHAEL/AU
E9          126      OLIVE DANIEL/AU
E10          14      OLIVE DAVID/AU

=> more
E11          11      OLIVE DAVID I/AU
E12          20      OLIVE DAVID L/AU
E13           2      OLIVE DEBORAH L/AU
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E21           2      OLIVE G CHAPMAN/AU
E22           2      OLIVE G HENRICI/AU

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L55          48 FILE CAPLUS

TOTAL FOR ALL FILES
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L57           0 FILE CA
L58           0 FILE CAPLUS

TOTAL FOR ALL FILES
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L60           3 FILE CA
L61           3 FILE CAPLUS

TOTAL FOR ALL FILES
L62           6 L50 AND (L53 OR L56)


L63           0 FILE CA
L64           0 FILE CAPLUS

TOTAL FOR ALL FILES
L65           0 L53 AND L56


SEARCH ENDED BY USER
SEARCH ENDED BY USER

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L67           3 FILE CAPLUS

TOTAL FOR ALL FILES
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PROCESSING COMPLETED FOR L68
L69           3 DUP REM L68 (3 DUPLICATES REMOVED)
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L69 ANSWER 1 OF 3 CA COPYRIGHT 1996 ACS DUPLICATE 1
AN 122:259841 CA
TI 5'-nucleases derived from thermostable DNA polymerases and their use in a nucleic acid detection method
IN Dahlberg, James E.; **Lyamichev, Victor I.; Brow, Mary Ann D.**
PA Third Wave Technologies, Inc., USA
SO PCT Int. Appl., 158 pp.
CODEN: PIXXD2
PI WO 9429482 A1 941222
DS W: AU, CA, JP
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE
AI WO 94-US6253 940606
PRAI US 93-73384 930604
DT Patent
LA English
AB Derivs. of thermostable DNA polymerases that retain their 5'-nuclease activity but lack polymerase are described for use in a nucleic acid detection system. The nuclease activity cleaves the single-stranded moiety of a Y-shaped structure and so is of use in selected cleavage of reporter sequences in a hybridization assay that includes two 5'-nuclease-dependent cleavage and amplification steps. The presence of the target sequence is demonstrated by the release of the reporter moiety from sequences immobilized on a carrier. The ability of the nuclease activity to cleave such structures was shown by the inability of intact Taq polymerase to amplify a hairpin sequence, although the nuclease-free Stoffel fragment could amplify the target sequence. The prepn. and characterization of a no. of polymerase mutants for use is in these assays is demonstrated.
IT Thermus aquaticus
Thermus flavus
Thermus thermophilus
(DNA polymerase of; 5'-nucleases derived from thermostable DNA polymerases and their use in nucleic acid detection method)
IT Nucleic acid hybridization
(cleavage amplification reaction for detection of hybridization in; 5'-nucleases derived from thermostable DNA polymerases and their use in nucleic acid detection method)
IT Deoxyribonucleic acid sequences
(for Taq polymerase analogs lacking polymerase activity but retaining nuclease activity; 5'-nucleases derived from thermostable DNA polymerases and their use in nucleic acid detection method)
IT Gene, microbial
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(for Taq polymerase and polymerase-deficient analogs; 5'-nucleases derived from thermostable DNA polymerases and their use in nucleic acid detection method)
IT Protein sequences
(of Taq polymerase analogs lacking polymerase activity but retaining nuclease activity; 5'-nucleases derived from thermostable DNA polymerases and their use in nucleic acid

detection method)

IT 79393-91-2P, Cleavase BX
RL: ARG (Analytical reagent use); BMF (Bioindustrial manufacture); ANST (Analytical study); BIOL (Biological study); PREP (Preparation); USES (Uses)
(5'-nucleases derived from thermostable DNA polymerases and their use in nucleic acid detection method)

IT 37337-14-7P, 5'-Endonuclease
RL: ARG (Analytical reagent use); BPN (Biosynthetic preparation); ANST (Analytical study); BIOL (Biological study); PREP (Preparation); USES (Uses)
(5'-nucleases derived from thermostable DNA polymerases and their use in nucleic acid detection method)

IT 9012-90-2D, DNA polymerase, amino acid-substituted analogs
RL: MSC (Miscellaneous)
(5'-nucleases derived from thermostable DNA polymerases and their use in nucleic acid detection method)

IT 123340-12-5 162393-95-5
RL: ARG (Analytical reagent use); BUU (Biological use, unclassified); PRP (Properties); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(amino acid sequence; 5'-nucleases derived from thermostable DNA polymerases and their use in nucleic acid detection method)

IT 162393-93-3 162393-94-4 162393-96-6 162393-97-7 162393-98-8 162393-99-9 162394-00-5 162394-01-6 162394-02-7
RL: ARG (Analytical reagent use); BUU (Biological use, unclassified); PRP (Properties); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(nucleotide sequence; 5'-nucleases derived from thermostable DNA polymerases and their use in nucleic acid detection method)

L69 ANSWER 2 OF 3 CA COPYRIGHT 1996 ACS DUPLICATE 2
AN 121:75320 CA
TI Site-directed cleavage of nucleic acids using pilot oligonucleotides
IN Dahlberg, James E.; **Lyamichev, Victor I.; Brow, Mary Ann D.**
PA Wisconsin Alumni Research Foundation, USA
SO Eur. Pat. Appl., 22 pp.
CODEN: EPXXDW
PI EP 601834 A1 940615
DS R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE
AI EP 93-309827 931207
PRAI US 92-986330 921207
DT Patent
LA English
AB A method of cleaving a target nucleic acid mol. by use of an oligonucleotide with two domains is described. One of these domains is complementary to a sequence 5' or 3' to the cleavage site and the other domain is not complementary to the target DNA. Upon hybridization a Y-shaped complex is formed exposing the junction site for cleavage, e.g. with a nuclease. Suitable enzymes for cleaving at the junction include the thermostable nuclease

activities of DNA polymerase such as Taq, Tfl, Tth, and non-thermostable polymerases such as the Escherichia coli enzyme and the gene 6 protein of bacteriophage T7. The presence of a 5'-exonuclease activity in Taq polymerase is demonstrated and the enzyme is used to cleave a PCR amplification product.

IT Deoxyribonucleic acids
Nucleic acids
Ribonucleic acids
RL: BIOL (Biological study)
(site-directed cleavage of, formation of single-stranded junction with pilot oligonucleotides in)

IT Thermus aquaticus
Thermus flavus
Thermus thermophilus
(thermostable DNA polymerase of, 5'exonuclease activity of, for cleavage of nucleic acids at single-stranded/double-stranded junctions, formation of junctions with oligonucleotides in)

IT Proteins, specific or class
RL: BIOL (Biological study)
(gene 6, for cleavage of nucleic acids at single-stranded/double-stranded junctions, formation of junctions with oligonucleotides in)

IT Nucleotides, biological studies
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(oligo-, forming partially single-stranded hybrids with a target nucleic acid, in site-directed cleavage)

IT 9012-90-2, DNA polymerase
RL: USES (Uses)
(thermostable, 5'-exonuclease activity of, for cleavage of nucleic acids at single-stranded/double-stranded junctions, formation of junctions with oligonucleotides in)

IT 9025-82-5, 5'-Exonuclease
RL: USES (Uses)
(thermostable, of DNA polymerases, for cleavage of nucleic acids at single-stranded/double-stranded junctions, formation of junctions with oligonucleotides in)

L69 ANSWER 3 OF 3 CA COPYRIGHT 1996 ACS DUPLICATE 3
AN 119:176439 CA
TI Structure-specific endonucleolytic cleavage of nucleic acids by eubacterial DNA polymerases
AU **Lyamichev, Victor; Brow, Mary Ann D.**; Dahlberg, James E.
CS Sch. Med., Univ. Wisconsin, Madison, WI, 53706, USA
SO Science (Washington, D. C., 1883-) (1993), 260(5109), 778-83
CODEN: SCIEAS; ISSN: 0036-8075
DT Journal
LA English
AB Previously known 5' exonucleases of several eubacterial DNA polymerases have now been shown to be structure-specific endonucleases that cleave single-stranded DNA or RNA at the bifurcated end of a base-paired complex. Cleavage was not coupled

to synthesis, although primers accelerated the rate of cleavage considerably. The enzyme appeared to gain access to the cleavage site by moving from the free end of a 5' extension to the bifurcation of the duplex, where cleavage took place. Essentially any linear single-stranded nucleic acid can be targeted for specific cleavage by the 5' nuclease of DNA polymerase through hybridization with an oligonucleotide that converts the desired cleavage site into a substrate.

IT Deoxyribonucleic acids
RL: BIOL (Biological study)
(endonucleolytic cleavage of single-stranded, by DNA polymerase-assocd. exonuclease of bacteria, 5' end structure effect on)

IT Ribonucleic acids
RL: RCT (Reactant)
(endonucleolytic cleavage of, by DNA polymerase-assocd. exonuclease of bacteria, 5' endstructure in relation to)

IT Bacteria
(exonuclease assocd. with DNA polymerase of, structure-specific endonucleolytic cleavage reactions of)

IT Molecular structure-biological activity relationship
(nuclease substrate, of single-stranded RNA and DNA)

IT 9025-82-5, 5'-Exonuclease
RL: BIOL (Biological study)
(DNA polymerase-assocd., of bacteria, structure-specific endonucleolytic cleavage reactions of)

IT 7439-95-4, Magnesium, biological studies
RL: BIOL (Biological study)
(RNA endonucleolytic cleavage by DNA polymerase-assocd. exonuclease of bacteria dependence on)

IT 150340-87-7
RL: RCT (Reactant)
(endonucleolytic cleavage of, by DNA polymerase-assocd. exonuclease of bacteria in primer presence and absence, 5'-end structure in relation to)

IT 150340-89-9 150340-90-2 150340-91-3 150340-92-4
RL: RCT (Reactant)
(endonucleolytic cleavage of, by DNA polymerase-assocd. exonuclease of bacteria, 5'-end structure relation to)

IT 9012-90-2, DNA polymerase
RL: BIOL (Biological study)
(exonuclease assocd. with, of bacteria, structure-specific endonucleolytic cleavage reactions of)

IT 150340-88-8
RL: BIOL (Biological study)
(single-stranded DNA hairpin endonucleolytic cleavage by DNA polymerase-assocd. exonuclease of bacteria in presence of)

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L72          10 FILE EMBASE
L73          12 FILE LIFESCI
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L78          13 FILE SCISEARCH

TOTAL FOR ALL FILES
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TOTAL FOR ALL FILES
L89         125 LYAMICHEV V?/AU


L90         325 FILE BIOSIS
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L93          81 FILE LIFESCI
L94           4 FILE BIOTECHDS
L95           8 FILE WPIDS
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L97           6 FILE DISSABS
L98         434 FILE SCISEARCH

TOTAL FOR ALL FILES
L99        1426 OLIVE D?/AU

=> s l79 and l89 and l99; s l79 and (l89 or l99); s l89 and l99
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L101          0 FILE MEDLINE
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L103          0 FILE LIFESCI
L104          0 FILE BIOTECHDS
L105          0 FILE WPIDS
L106          0 FILE CONFSCI
L107          0 FILE DISSABS
L108          0 FILE SCISEARCH

TOTAL FOR ALL FILES
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L111          1 FILE MEDLINE
L112          1 FILE EMBASE
L113          1 FILE LIFESCI
L114          1 FILE BIOTECHDS
L115          2 FILE WPIDS
L116          0 FILE CONFSCI
L117          0 FILE DISSABS
L118          1 FILE SCISEARCH

TOTAL FOR ALL FILES
L119          8 L79 AND (L89 OR L99)


L120          0 FILE BIOSIS
L121          0 FILE MEDLINE
L122          0 FILE EMBASE
L123          0 FILE LIFESCI
L124          0 FILE BIOTECHDS
L125          0 FILE WPIDS
L126          0 FILE CONFSCI
L127          0 FILE DISSABS
L128          0 FILE SCISEARCH

TOTAL FOR ALL FILES
L129          0 L89 AND L99
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=> s l119 not (l19 or l46)
L130          1 FILE BIOSIS
L131          1 FILE MEDLINE
L132          1 FILE EMBASE
L133          1 FILE LIFESCI
L134          1 FILE BIOTECHDS
L135          2 FILE WPIDS
L136          0 FILE CONFSCI
L137          0 FILE DISSABS
L138          1 FILE SCISEARCH

TOTAL FOR ALL FILES
L139          8 L119 NOT (L19 OR L46)

=> dup rem l139
PROCESSING COMPLETED FOR L139
L140           3 DUP REM L139 (5 DUPLICATES REMOVED)

=> d 1-3 bib abs; fil hom

L140  ANSWER 1 OF 3  BIOTECHDS COPYRIGHT 1996 DERWENT INFORMATION LTD
AN    95-03012  BIOTECHDS
TI    5'-Nucleases derived from thermostable DNA-polymerases;
         having cleavage activity without interfering synthetic ability,
         and methods using the nucleases for detection of specific target
         sequences
AU    Dahlberg J E; Lyamichev V I; Brow M A D
PA    Third-Wave-Technol.
PI    WO 9429482 22 Dec 1994
AI    WO 94-US6253 6 Jun 1994
PRAI  US 93-73384 4 Jun 1993
DT    Patent
LA    English
OS    WPI: 95-036504 [05]
AN    95-03012  BIOTECHDS
AB    A DNA sequence (I) encoding a thermostable DNA-polymerase (DP,
      EC-2.7.7.7) altered in sequence relative to the native sequence
      such that it exhibits altered DNA synthetic activity from that of
      the native DP is new. Also claimed are: i. a recombinant DNA vector
      containing (I); ii. a host cell transformed with the vector; iii. a
      thermostable DP altered in amino acid sequence such that it
      exhibits altered DNA synthetic activity from that of the native DP,
      but retains substantially the same 5' nuclease activity; and iv.
      methods for detecting the presence of a specific target DNA
      molecule.  The new thermostable DP retains 5'-nuclease activity
      while the synthetic activity is reduced or absent.  These new
      properties form the basis of a method of detecting specific nucleic
      acid sequences.  The method relies upon the amplification of the
      detection molecule rather than on the amplification of the target
      sequence itself as do existing methods.  The alteration to the
      native sequence is a single nucleotide deletion or insertion.
      Examples of DP sequences altered by deletion are specified.  The
```

DNA is preferably derived from Thermus aquaticus, Thermus flavus or Thermus thermophilus. (159pp)

L140 ANSWER 2 OF 3 WPIDS COPYRIGHT 1996 DERWENT INFORMATION LTD
AN 94-185200 [23] WPIDS
DNC C94-083894
TI A method of cleaving a nucleic acid mol. at a specific target site - comprising forming a cleavage structure exposing this to a cleavage agent and incubating.
DC B04 D16
IN **BROW, M A D**; DAHLBERG, J E; **LYAMICHEV, V I**
PA (WISC) WISCONSIN ALUMNI RES FOUND
CYC 19
PI EP 601834 A1 940615 (9423)* EN 22 pp
R: AT BE CH DE DK ES FR GB GR IE IT LI LU MC NL PT SE
JP 06303975 A 941101 (9503) 16 pp
US 5422253 A 950606 (9528) 18 pp
ADT EP 601834 A1 EP 93-309827 931207; JP 06303975 A JP 93-339818 931207; US 5422253 A US 92-986330 921207
PRAI US 92-986330 921207
AN 94-185200 [23] WPIDS
AB EP 601834 A UPAB: 940727
Cleaving a target nucleic acid (I) at a specific target site comprises: (a) forming a cleavage structure comprising (I) and a pilot nucleic acid (II), where a first region of (I) is annealed to (II) to form a duplex and where a second region of (I) contiguous to the duplex is not annealed to (II), forming a junction site between the duplex region and the non-annealed region; and (b) exposing the cleavage structure to a cleavage agent capable of preferentially cleaving the cleavage structure at a target site in a manner independent of the sequence of the cleavage structure; and (c) incubating the cleavage structure and cleavage agent under conditions where cleavage can occur.

USE - Using the method it is possible to manipulate nucleic acid mols. In particular a 5 '-exonuclease activity of a DNA polymerase is used cleave a nucleic acid mol. The method is used to cleave a mol. in which there is some internal homology so that a part of the mol. will anneal with another part of the mol.. This is useful to detect internal sequence differences in DNA fragments without prior knowledge of the specific sequences of the variants. Dwg.0/0

ABEQ US 5422253 A UPAB: 950721
Cleavage of single-stranded nucleic acid at a specific site comprises construction of a model nucleic acid having a sequence that is complementary to that of one side of the cleavage site; prepn. of a cleavage structure of the given nucleic acid and the model, such that the model is free from any region not annealed to the given nucleic acid, but the sequence on one side of the cleavage site of the given nucleic acid is annealed to the model to form a duplex, but the sequence in the other side of the cleavage site in the given nucleic acid is not annealed to the model; and incubating the cleavage structure with either a DNA polymerase having 5'-nuclease activity, or with a gene-6-prod. from bacteriophage T-7.

USE - The process facilitates nucleic acid analysis and identification.

ADVANTAGE - The process allows cleavage at sites contg. sequences not normally cleaved by restriction endonucleases. Dwg.0/10

L140 ANSWER 3 OF 3 BIOSIS COPYRIGHT 1996 BIOSIS DUPLICATE 2
AN 93:324385 BIOSIS
DN BA96:32735
TI STRUCTURE-SPECIFIC ENDONUCLEOLYTIC CLEAVAGE OF NUCLEIC ACIDS BY EUBACTERIAL DNA POLYMERASES.
AU **LYAMICHEV V; BROW M A D;** DAHLBERG J E
CS DEP. BIOMOLECULAR CHEM., UNIVERSITY WISCONSIN SCH. MED., 1300 UNIVERSITY AVE., MADISON, WI 53706, USA.
SO SCIENCE (WASHINGTON D C) 260 (5109). 1993. 778-783. CODEN: SCIEAS ISSN: 0036-8075
LA English
AB Previously known 5' exonucleases of several eubacterial DNA polymerases have now been shown to be structure-specific endonucleases that cleave single-stranded DNA or RNA at the bifurcated end of abase-paired duplex. Cleavage was not coupled to synthesis, although primers accelerated the rate of cleavage considerably. The enzyme appeared to gain access to the cleavage site by moving from the free end of a 5' extension to the bifurcation of the duplex, where cleavage took place. Single-stranded 5' arms up to 200 nucleotides long were cleaved from such a duplex. Essentially any linear single-stranded nucleic acid can be targeted for specific cleavage by the 5' nuclease of DNA polymerase through hybridization with an oligonucleotide that converts the desired cleavage site into a substrate.

FILE 'HOME' ENTERED AT 17:14:25 ON 08 MAY 96

=> fil ca,caplus; s (l50 or l53 or l56) and (l4 or taq polymerase#)
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L141 5 FILE CA
L142 5 FILE CAPLUS

TOTAL FOR ALL FILES
L143 10 (L50 OR L53 OR L56) AND (L4 OR TAQ POLYMERASE#)

=> s l143 not (l8 or l25 or l68)
L144 1 FILE CA
L145 1 FILE CAPLUS

TOTAL FOR ALL FILES
L146 2 L143 NOT (L8 OR L25 OR L68)

=> dup rem l146
PROCESSING COMPLETED FOR L146
L147 1 DUP REM L146 (1 DUPLICATE REMOVED)

=> d .bevstr1; fil
biosi,medl,embas,lifesci,biotechds,wpids,confsci,dissabs,scisearch

L147 ANSWER 1 OF 1 CA COPYRIGHT 1996 ACS DUPLICATE 1
AN 113:127722 CA
TI Methods for DNA sequencing with (Thermus **aquaticus**) **DNA polymerase**
IN Innis, Michael A.; Myambo, Kenneth B.; Gelfand, David H.; **Brow, Mary Ann D.**
PA Cetus Corp., USA
SO PCT Int. Appl., 29 pp.
CODEN: PIXXD2
PI WO 9003442 A1 900405
DS W: AU, JP
RW: AT, BE, CH, DE, FR, GB, IT, LU, NL, SE
AI WO 89-US4093 890919
PRAI US 88-249367 880923
DT Patent
LA English
AB Methods and conditions for DNA sequencing by the dideoxy method using **Taq polymerase** are described. The high optimal temp. of this enzyme is useful for the sequencing of GC-rich DNA. The enzyme will also use secondary structure-disrupting nucleotide analogs (ITP, deazaguanine triphosphate), and the amplification products from polymerase chain reaction can be directly sequenced. Sequences >1000 nucleotides long are detd. in a single set of sequencing reactions.
IT Thermus aquaticus
(thermostable DNA polymerase from, dideoxy DNA sequencing using)
IT 365-08-2 1927-31-7 2056-98-6 2564-35-4 16595-02-1 101515-08-6
RL: PRP (Properties)
(as thermostable **Taq polymerase** substrate in DNA sequencing by dideoxy method)
IT 9012-90-2
RL: BIOL (Biological study)
(Taq, thermostable, DNA sequencing by dideoxy method using, elevated temp. in)

FILE 'BIOSIS' ENTERED AT 17:17:16 ON 08 MAY 96

FILE 'MEDLINE' ENTERED AT 17:17:16 ON 08 MAY 96

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FILE 'EMBASE' ENTERED AT 17:17:16 ON 08 MAY 96
COPYRIGHT (C) 1996 Elsevier Science B.V.  All rights reserved.

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FILE 'WPIDS' ENTERED AT 17:17:16 ON 08 MAY 96
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FILE 'CONFSCI' ENTERED AT 17:17:16 ON 08 MAY 96
COPYRIGHT (C) 1996 Cambridge Scientific Abstracts (CSA)

FILE 'DISSABS' ENTERED AT 17:17:16 ON 08 MAY 96
COPYRIGHT (C) 1996 University Microfilms, Inc.

FILE 'SCISEARCH' ENTERED AT 17:17:16 ON 08 MAY 96
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=>  s (l79 or l89 or l99) and (l4 or taq polymerase#)
L148          2 FILE BIOSIS
L149          3 FILE MEDLINE
L150          2 FILE EMBASE
'CN' IS NOT A VALID FIELD CODE
L151          2 FILE LIFESCI
L152          1 FILE BIOTECHDS
'CN' IS NOT A VALID FIELD CODE
L153          1 FILE WPIDS
'CN' IS NOT A VALID FIELD CODE
L154          0 FILE CONFSCI
'CN' IS NOT A VALID FIELD CODE
L155          0 FILE DISSABS
'CN' IS NOT A VALID FIELD CODE
L156          1 FILE SCISEARCH

TOTAL FOR ALL FILES
L157         12 (L79 OR L89 OR L99) AND (L4 OR TAQ POLYMERASE#)

=> s l157 not (l19 or l46 or l139)
L158          1 FILE BIOSIS
L159          1 FILE MEDLINE
L160          1 FILE EMBASE
L161          1 FILE LIFESCI
L162          0 FILE BIOTECHDS
L163          0 FILE WPIDS
L164          0 FILE CONFSCI
L165          0 FILE DISSABS
L166          1 FILE SCISEARCH

TOTAL FOR ALL FILES
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L167 5 L157 NOT (L19 OR L46 OR L139)

=> dup rem l167
PROCESSING COMPLETED FOR L167
L168 3 DUP REM L167 (2 DUPLICATES REMOVED)

=> d 1-3 bib abs; fil hom

L168 ANSWER 1 OF 3 LIFESCI COPYRIGHT 1996 CSA
AN 91:69900 LIFESCI
TI Methods for DNA sequencing with Thermus **aquaticus DNA polymerase.**
AU Innis, M.A.; Myambo, K.B.; Gelfand, D.H.; **Brow, M.A.D.**
CS Cetus Corporation, Emeryville, CA (USA)
PI US 4075216 1991
SO (1991) . US Cl. 435/6; Int. Cl. C12P 19/34..
DT Patent
FS Q4; W; A
LA English

L168 ANSWER 2 OF 3 BIOSIS COPYRIGHT 1996 BIOSIS DUPLICATE 1
AN 89:161249 BIOSIS
DN BA87:83350
TI DETECTION OF ENTEROTOXIGENIC ESCHERICHIA-COLI AFTER POLYMERASE CHAIN REACTION AMPLIFICATION WITH A THERMOSTABLE DNA POLYMERASE.
AU **OLIVE D M**
CS DEP. MICROBIOL., FAC. MED., KUWAIT UNIV., P.O. BOX 24923, 13110 SAFAT, KUWAIT.
SO J CLIN MICROBIOL 27 (2). 1989. 261-265. CODEN: JCMIDW ISSN: 0095-1137
LA English
AB The direct identification of enterotoxigenic Escherichia coli from clinical specimens was examined by using the polymerase chain reaction (PCR) for amplifying the heat-labile toxin (LT) gene. Two synthetic primers, each of which was 20 bases in length, were used with the thermostable DNA polymerase from Thermus aquaticus to amplify the LT gene. The amplified PCR products were detected by either gel electrophoresis or hybridization to a 24-base synthetic oligonucleotide probe conjugated to alkaline phosphatase. The PCR method detected LT-positive bacteria but did not react with E. coli producing the heat-stable toxin, enteroinvasive E. coli, Salmonella typhi, Salmonella typhimurium, or Shigella dysenteriae. By the PCR method, a single bacterium could be detected following 30 cycles of amplification. The T. **aquaticus DNA polymerase** was inhibited by more than 103 organisms in the amplification reaction mixture. A group of 40 clinical specimens consisting of 16 LT bioassay-positive and 24 LT bioassay-negative stool specimens were tested by PCR for the presence of toxigenic E. coli. The total DNA from 100 .mu.l of stool specimen was extracted and partially purified with a commercially available ion-exchange column. All 16 of the bioassay-positive stool specimens were positive by PCR. In addition, one stool specimen which was bioassay negative for LT but positive for LT in a previous hybridization assay with a

different LT probe was also positive by PCR. This may indicate that the LT gene is present but either is not expressed or is expressed below detectable levels. Amplification of specific DNA sequences by PCR provides a highly sensitive and specific tool for the detection of pathogenic microorganisms directly from clinical specimens without the need for prior isolation. This technique may find wide application in the detection of other organisms in addition to enterotoxigenic E. coli.

L168 ANSWER 3 OF 3 SCISEARCH COPYRIGHT 1996 ISI (R)
AN 89:4354 SCISEARCH
GA The Genuine Article (R) Number: R5187
TI DNA SEQUENCING WITH THERMUS-**AQUATICUS DNA-POLYMERASE** AND DIRECT SEQUENCING OF POLYMERASE CHAIN REACTION-AMPLIFIED DNA
AU INNIS M A (Reprint); MYAMBO K B; GELFAND D H; **BROW M A D**
CS CF MENNINGER MEM HOSP, DEPT MICROBIAL GENET, 1400 53RD ST, TOPEKA, KS, 66601 (Reprint)
CYA USA
SO PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1988) Vol. 85, No. 24, pp. 9436-9440.
DT Article; Journal
FS LIFE
LA ENGLISH
REC Reference Count: 19

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=> e "7-deaza-datp"/cn 5
E1            1      7-DEAZA-8-AZAGUANOSINE/CN
E2            1      7-DEAZA-AMP/CN
E3            0 --> 7-DEAZA-DATP/CN
E4            1      7-DEAZAADENINE/CN
E5            1      7-DEAZAADENOSINE/CN

=> fil ca,caplus
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